DETERMINATION OF FETAL LUNG MATURITY BY LECITHINSPHINGOMYELIN RATIO IN AMNIOTIC FLUID

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CERTIFICATE

This is to certify that work on "DETERMINATION OF FOETAL LUNG MATURITY BY LECITHIN-SPHINGOMYELIN RATIO IN AMNIOTIC FLUID", by Dr. Subhpreet Kaur Bhullar has been done in the Department of Obstetrics & Gynaecology, M.L.B. Medical College, Jhansi under my guidance and supervision.

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C E R T I F I C A T E

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(SUKHDEEM KOUR BHULLAR)

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I N T R O D U C T I O N

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Considerable attention is now being paid to foetal maturity and to methods for its assessment, not only by obstetricians and neonatologists but their colleagues, providing diagnostic support in Biochemistry, Radiology or ultrasonics.

Occassionally few cases of fulminent pre-eclampsia and placental abruption need induction in mother's interest but many cases of dismaturity, elderly primigravidas and mothers - with bad obstetrical history are allowed to continue pregnancy to as near term as possible, although these are high risk patients.

In certain cases of post maturity labour is often induced under an erroneous impression of the diagnosis. Besides if induction fails in these cases specially if the nature of induction was artificial rupture of membranes, intra-uterine injection supervenes and caesarean section complicates the matter further.

In all these circumstances, careful assessment of foetal maturity is essential. Therapy so far reasons like bad memory and uncertain timing of ovulation specially soon after oral contraception and lactational amenorrhoea where menstrual history is often an unreliable guide for calculation of gestation age.

For the obstetrician who has reasons to terminate a pregnancy the assessment of foetal maturity and also its intrauterine state is of great importance.

Foetal maturity includes the simple chronological process of increasing gestational age, the growth of the foetus in terms of increasing size and weight, and functional maturity signifying physiological development of the foetal tissues and systems. Latter is the most important amongst these which determines viability of the foetus. It is upon the functional capacity of the lungs, rather than other organs, that the undamaged live born babyds survival depends.

Since it is clear that single determinant of the foetal capacity for extra uterine viability relates to his capacity to emerge from a watery environment ready and able to breath air, the most practical and meaningful measure of foetal maturity would be the ability to determine whether a foetus could successfully meet this supreme challenge of extrauterine environment.

Prematurity with low birth weight is one of the main etiological factors in perinatal mortality. Since the preterm infant is subjected to the risk of hyaline membrane disease causing respiratory distress and this complication is a major cause of death in these small infants.

The lungs of these neonates are deficient in surfactant, which because of its unique variable surface tension effect when compressed prevents atelectasis and collapse of the alveoli at the end of expiration, thereby maintaining expansion of the alveoli on inspiration.

Babies born without this protective coating may developed respiratory distress syndrome (R.D.S). In this situation, alveolar surface will be elevated after expiration causing alveoli to collapse and inducing progressive atelectasis.

Gluck (1967) has found that this surface active substance is abundant in neutral lipids and phospholipids specially lecithin, perhaps lecithin rich material could be detected before term by amniocentesis because it has been suggested that foetal tracheobronchial tree contributes in part to the contents of amniotic fluid. The relative proportions of lecithin and sphingomyelin in amniotic fluid analysis proved diagnostic to maturity. Prior to alveolar stability (about 35 weeks gestation) the ratio of lecithin to sphingomyelin is less than or equal to 1 (one). Pulmonary maturity, however, was heralded by a sudden change in the ratio in favour of lecithin. A ratio more than 2:1 indicates that a baby born at that point would not develop respiratory distress syndrome.

As literary data are quite unreliable and even contradictory it si of interest to handle this problem again particularly in large series doses of normal and abnormal pregnancies with following aims and objectives :-

(1) To estimate levels of lecithin and sphingomyelin and their ratio during various periods of gestation.

(2) To see whether amniotic fluid L-S ratio can serve as a good parameter for foetal lung maturity.

(3) To see whether L/S ratio can be of significance in complicated pregnancies in assessing foetal lung maturity.

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REVIEW OF LITERATURE

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Foetal respiratory tract and amniotic fluid :-

Amongst several theories of amniotic fluid origin and turnover, the role of foetal lungs in its secretion and absorption is most plausible.

Reynolds's (1953) view of secretion from nasopharynx in new born lamb was further supported by Enhorning and Adams (1963). They reported that 0.02-0.013 ml/Kg per minute of fluid is produced in respiratory tract of foetus. Adams et al (1965) showed that the alveolar phospholipid levels equated with lung tissue levels.

Tower (1966) believed no pulmonary contribution, but Moller (1969) demonstrated relatively high chloride ion content of amniotic fluid attributable to pulmonary secretion. Goodlin and Randolph (1970) suggested initial higher volumes of bronchial secretion after rapid deliveries.

Biggs and Duncan (1970) suggested the foetal respiratory tract secretions to be the additional source in the later part of pregnancy. It has also been postulated that fluid passes from lungs to amniotic cavity (Gluck and Whitfield, 1971); Bhagwanani et al (1972). Foetal lungs contribute fluid and surface active lecithin to amniotic fluid in late pregnancy (Biggs and McCeory 1973).

Scarpelli (1975) studied entrance of albumine in liquor through respiratory tract. The view of Duenhoelter and Switchard (1976) for foetal lung absorption capacity was further supported by Whitfield (1976), that intrauterine breathing movements result in small tidal flow in liquor allowing absorption by lungs as well as access of pulmonary secretions to the amniotic fluid.

Foetal pulmonary maturation and surfactant production :-

The initial development of the bronchial tree, from the appearance of the endodermal lung buds (at 24 days of foetal life) to the formation of terminal conducting bronchioles (at 16 weeks), is followed by an intermediate phase during which the respiratory bronchioles are formed, and then by final stage of alveolar development which begins at about 24 weeks and continues beyond birth into postnatal life. During the final phase, the alveolar ducts and sacs form, the alveolar lining membrane differentiates into type I and type II cells, the pneumocytes. The pulmonary surfactant is produced by type II cells.

It is the appearance of surfactant at the start of tthis final phase that marks the begining of functional pulmonary development or maturation, and makes possible the maintenance of alveolar expension and ventilation, so that, in the event of birth survival is now possible .

Surface tension is the force acting to reduce the area of a surface. Its effect on the specially curved alveolar surfaces is to inhibit distension (Primary atelectasis), to promote collapse of alveoli that have been distended (secondary atelectasis), and to resist reinflation. By studying the lung washing film, Clements (1957) suggested that the stability of alveoli during expiration is due to this property.

Clements et al (1957) identified the presence of surfactant in the lung tissue. The concept of Pattle (1958) of deficient surface active material in the alveolar lining as a cause of progressive atelectasis of hyaline membrane disease, was confirmed by Avery and Mead (1959). The active component of this alveolar surfactant the phospholipid lecithin, and of less importance sphingomyelin are present in the lungs of a very premature infants (Adams et al ; 1955), but the there is progressive increase in the amount towards term (Gluck et al ; 1967).

The terminal increase of phospholipids steadily improves the distensibility of lungs, and by providing an excess or preservoir of surfactant by the time of birth, under normal conditions it virtually eliminates the risk of R.D.S. (Brumley et al (1967).

Thus despite other factors in the aetiology of R.D.S and hyaline membrane diseases, the primary facto factor is insufficiency of surfactant on the alveolar surfaces, due to its inadequate synthesis, release or a replenishment or a combination of these by the type II cells. Foetal lungs contribution to phospholipid constituents (Scrapelli , 1967 ; Nelson, 1969) was further supported by Goodlin and Rudolph (1970) that foetal lungs are one of the some source of amniotic fluid origin.

Gluck et al (1971) by biochemical proceedings con firmed that surface active lecithin in the amniotic fluid at birth and in the fluid from new-born trachea are identical, and terminal increase in foetal lung lecithin is reflected by rising con centration in amniotic fluid, so the amount of surface active lecithin in amniotic fluid provides functional maturation index of foetal lungs. Morgan (19 71) demonstrated the release of lecithin from inclusion bodies of alveolar type II cells. Studying 400 premature and mature babies, Gluck et al (1972) considered 3 locally inhibitory vicious circles to surfactant production in R.D.S. These are namely the formation of an alveolar exudate (from which hya line membranes themselves are formed) due to raised negative intrathoracic pressure, diminished

pulmonary blood flow resulting from hypoxia and acidos acidosis, and further reduction of pulmonary blood flow as a direct effect of the hyaline membranes.

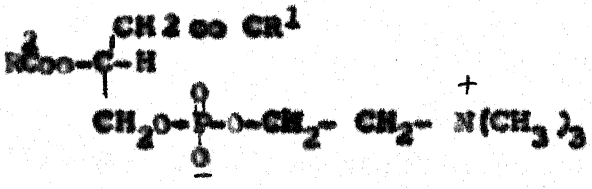
From the same study Gluck et al (1972) identified two separate pathways for lecithin biosynth -esis in amniotic fluid in the human foetal lung. Pathway I- relatively simple and responsible for the surge in the alveolar surface activity towards term is the main pathway. By choline incorporation leading to dipalmitoyl lecithin molecule (α Palmitic : β - Palmitic fraction). Pathway II- is the main source of surfactant before active terminal phase of pathway I occurs, is the methylation of phosphatidyl ethenolamine to form palmitic palmitic : B-myristic lecithin, also described as a marginal pathway, is likely to be inhibited by fa ctors such as - acidosis, hypoxia and hypothermia. But Hallman & Gluck, (1974) disproved the pathway II stating that phosphatidyl glycerol, a major su rface active phospholipid in adult lung, had been mistaken for phosphatidyl dimethyl-ethenolamine. H allman et al (1975) observed that phosphatidyl glycerol appeared during recovery phase of R.D.S. whic which indicates that it has also some importance as a surfactant in foetal and neonatal lungs.

CHEMISTRY OF SURFACTANT

The barrier of the uterus has been broken by the introduction of amniocentesis (Bevis, 1953), and the foetus became accessible for information about foetal prognosis. This has proved highly beneficial to prevent perinatal mortality caused by prematurity due to deficiency of lung surfactant (Avery and Mead 1959). After intensive research the surfactant was known to be a complex lipoprotein (Pattle and Thomas, 1961) identified lecithin in the surface active fraction derived from beef lung. The respiratory distress, a major cause of death in small infants (Butler and Banham, 1963), was subsequently investigated for biochemical nature of pulmonary surfactant by Gluck et al (1967) and detailed study of elaboration of surfactant in the foetal lung was made.

Although phospholipids in amniotic fluid had been detected earlier (Nieszenski et al 1968; Nelson, 1969), it was Gluck et al (1971) to concentrate on the measurment of the lecithin in amniotic fluid and demonstrated that when the foetus becomes mature from the respiratory function point of view, there is a corresponding surge of lecithin into the amniotic fluid. Further more this rise reflects a chang in the metabolism of lecithin in the foetal lung to produce a molecule with greater surface activity (dipalmitoyl lecithin).

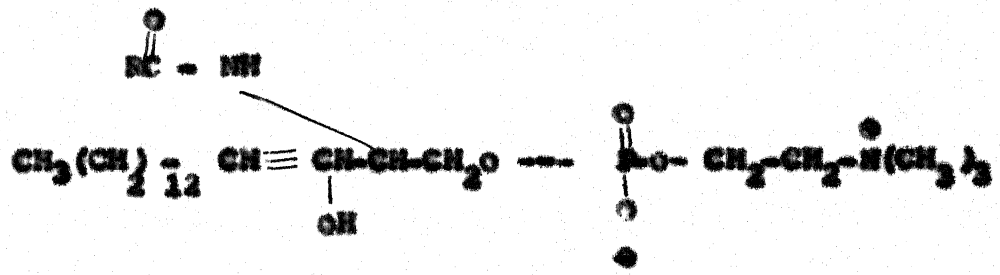
The major component of surfactent is a phospholipid i.e. esters of fatty acids with alcohol which contain another group. Only two groups of the phospholipids are considered (I) Glycerophospholipds with glycerol as alcohol built around a 3- carbon chain derived from glycerol. The addition of an organic base choline to the phosphatidic acid yields the resulting molecules of lecithin.



The molecule may be refferred to alternatively as lecitin L-α- lecithin, L_3(Di) Phosphatidyl Choline, (R)^3 phosphatidyl Choline, depending upon which classification of phospholipids is being employed. Most commonly, reference is made to lecithin or phosphatidyl Choline. The molecule may be further referred to in tems of fatty acid reduces on C_1 and C_2. Hence, if both are palmitic acid (C_{16}) the molecule is referred to dipalmitoyl lecithin.

It is significant that in early pregnancy the lecithin derived from amniotic fluid is rich in palmitic acid on C_1 but tends to have other fattyacid on C_2 eg., (C_{18}). With increasing pulmonary maturity the proportion of dipalmitoyl lecithin increases.

Sphingomyelin , a Sphingo-phospholipid with spingosine as the alcohol (Ceramide - 1-Phosphoryl Choline or Sphingomyelin).



Like lecithin each molecule of sphingomyelin contains one atom of phosphorus and one Choline grouping.

This molecule is of importance because Gluck (1972) chose to use the Sphingomyelin in amniotic fluid as a reference compound. As the concentration of sphingomyelin in amniotic fluid remained reasonably constant throughout gestation (or dimishes slightly towards term) by expressing the amount of lecitin present in terms of a lecithin/ sphingomyelin ratio (L/S ratio), errors due to fluctations in amniotic fluid volume could in theory be overcome.

AMNIOTIC FLUID LIPIDS

Total Lipids - Following preliminary studies by Nelmy and Mack (1962), Niesenski, (1962) identified various phospholipid by T.L.C., and found a small increase in total lipid concentration between 26 weeks and term (12.16 - 15.38mg/100 ml of amniotic fluid), and most of this was due to an increase in the phospholipid (From 3.15 mg/100 ml. to 5.19 mg/100 ml. of amniotic fluid). The concentration of nonpolar lipids remained relatively constant. The major component of the

phospholipid fraction was phosphatidyl Choline (lecithin), which accounts for 65% of the total phospholipid at terms.

By a study in normal and abnormal pregnancies, Nelson (1969) observed a decreased percentage of lecithin in the phospholipid fraction of R.D.S in the neonate, hyaline membrane diseases, anencephaly, premature- twin delivery. Gusden and Waite (1972) assumed that Sphingomyelin is not denatured by alkali and lecithin forms about 70% of amniotic fluid phosphglycerolipids.

Arvidson et al (1972), using method of Bligh and Dyer for phospholipid extraction and phosphorus determination by method of Chen et al, concluded that in the first trimester and term there is approximately a two fold increase in the phospholipid concentration. Whereas lecithin accounted for 28 and 51% initially, increasing to 50 and 79% of total phospholipids at term. However, fell from 39-51% to 25-46%

Niemenäi (1973) observed a linear increase of total phospholipids towards term. Lecithin concentration increasing 65 folds from 0.44 mg/ 100 ml. of amniotic fluid at early pregnancy to 2.92 mg/100 ml. of amniotic fluid at term. So lecithin is the principal phospholipid of late pregnancy, whereas, sphingomyelin appeared to be the principal phospholipid of early pregnancy with a fall in concentration from 1.07 mg% in early pregnancy to 0.025 mg per 100 ml at term.

Schereyer et al (1974) detected sharp increase in total phospholipids after 36 weeks contradictory to gradual rise by others

Schirar et al (1975) reported accentuated increase after 36 weeks gestation, but widely scattered values rendering single estimation unpredictive.

Lecithin :- As lecithin concentration increases with gestation period, the amniotic fluid lecithin values may be related with clinical prognosis of the new born.

Bhagwansani, Fahmy and Turnbull (1972) extracted lipids from amniotic fluid and demonstrated lecithin concentration acceleration from 34 weeks gestation. Between 14 and 22 weeks lecithin concentration was in order of 2.0 mg per 100 ml rising to 4mg at 34 and 36 weeks and reaching to 12 mg per 100 ml at term (mean values). The authors correlated lecithin concentration less than 3.5 mg/ 100 ml with reppiratory difficulty in new born.

Gaeden and Waite's (1972) method of alkali hydrolysis for lecithin estimation was further modified by Bayer et al (1973). Niemenski (1973) recorded rise in lecithin concentration from 0.44 mg/100 ml in early pregnancy to 2.92mg/100 ml of amniotic fluid in late pregnancy.

The disparity reflects the reduction of phospholipid concentration in toto largely due to differences in the handling , extraction and isolation of phospholipids, but certain proceedures such as centrifugation and filteration (Wagstaff et al; 1974) may selectively reduce lecithin compared with sphingomyelin. Lecithin comprises almost 80% of the surfactant phospholipid, Thelma et al (1980).

Fatty acids :- The elegant papers by Gluck et al (1967) a,b, 1970) on biochemical development of surface activity. Expressed change in the lecithin configuration with pulmonary maturation characterized by increase in the percentage of palmitic acid (C_{16}:0) on both the alpha (C_1) and Beeta C_2 carbon atoms of the molecule.

During assessment of pulmonary maturity by means of L/S ratio, it was observed that in some cases with 'low' i.e. such levels that might be expected to correlate with hyaline membrane disease or R.D.S., the new born was in fact unaffected. It was just possible, however, that the lecithin in these infants might have been qualitatively of the mature configuration rich in dipalmitoyl lecithin. Conversely , where L/S ratio was normal in some cases, specially babies born to diabetic mothers, the babies were adversely affected. (Wagstaff and Bromham, 1973; Whitfield and Sproule, 1974). In this group the lecithin may have been of a less mature configuration due to persistance of pathways which are normally superceded with the development of pulmonary maturity.

Russell et al (1974) equate pulmonary maturity with a level of at least 20% palimitic acid. Total fatty acids derived from a total lipid extract were investigated (Warren et al, 1974; a Moore et al ; 1975; Schirar et al; 1975), and observations were made that lecithin isolated from mature aminotic fluid has a high palmitic acid content and also that lecithin is the major source of palmitic acid in amniotic fluid. Other workers investigated fatty acid composition from lecithin separately by T.L.C. (Arvidson et ; 1972; Roux et al, 1974; Russell et al 1974; Das et al;. 1975) and Hill, 1978).

Schirar et al (1975) found palmitic acid more than 100% of the total fatty acid yield after 36 weeks and a mean of 51% at term. Moore et al (1975) found that respiratory distress did not develop when palmitic acid content was in excess of 25 ug (microgram)/ ml.

Palmitic/stearic acid ratio :- The selection of sphingomyelin as the reference compound to lecithin has been criticised (Mierzenski, 1973; Nelson et al, 1973; Biggs et al, 1973) on the grounds that the ratio depends upon the level of sphingomyelin the source of which may be unrelated to pulmonary surfactant.

Ekelund et al (1973) and Schirar et al (1975) demonstrated differences in the fatty acid distribution of lecithin in normal full term infants and in infants actually developing hyaline membrane disease.

If qualitative and quantitative changes of lecithin molecule reflect pulmonary maturity, than the ratio of palmitic acid derived from lecithin to the other fatty acid derived from the same source is free of the objections raised for sphingomyelin as well as the technical factors affecting lecithin relatively. The second substance is stearic acid.

So palmitic -stearic acid ratio appears to correlate well with neonatal respiratory performance (Zuspan et al, 1975). P/S ratio of 3.5 indicates lecithin predominently of mature configuration (Schiraretal 1975). But no satisfactory technique is yet available for its detection and determination. So P/S ratio has added as another index of foetal lung maturity (O'Neil, 1978 and Aleindor et al 1979).

<u>Lecithin - Sphingomyelin ratio</u> :- Estimation of ratio between lecithin and sphingomyelin is a widely used, quick, reliable, simple and careful rather than highly skilled laboratory technique for surfactant - measurement and can serve as guide to foetal lung maturation.

So the determination of L/S ratio has proved to be the major technique employed so far for the measurement of surfactant in amniotic fluid and various methods have been advocated for this purposes, namely :-

(i) Gravimetric Method

(ii) Densitometric Method

(iii) Planimetric Method

(iv) Molar method derived from lecithin and sphingomyelin phosphorus values.

After detection of amniotic fluid lipids by Blemenski et al (1968) correlation of prematurity and respiratory distress with phospholipid constituent in amniotic fluid by Nelson (1969) it was Gluck et al (1971), who by means of reflectance densitometry concluded that terminal rise in amniotic fluid lecithin towards term is not matched by corresponding increase in sphingomyelin concentration.

All A simple yet accurate and reproducible proceedure for L/S ratio determination introduced by Rorer et al (1971) is based upon measurement (length x width) of chromotographed spots of lecithin and sphingomyelin area ratio (L.S.A.R.).

All investigators agreed that L/S ratio is a useful predictor for foetal pulmonary maturity except Nakamura et al (1971), who correlated it with gestational age rather than pulmonary maturity. Whitfield et al (1972) agreed the wide variation in normal values, time of onset and terminal

rise at 32-37 weeks of gestation. Spellacy and Buhi (1972) found a significant correlation in L/S ratio and infant birth weight.

Whitfield et al (1972) studied 2000 amniotic fluid samples by this planimetric method and found L/S ratio more than 2.0 in 460 cases.

By using charring and densitometric method Gluck and his team (1973) established the practical value of L/S ratio and the factors influencing it in normal and complicated pregnancies by a series of investigators carried out by modification of surface active phospholipids.

Gluck and Kulovich (1973) achieved 100% accuracy in predicting 30 instances in R.D.S. form 51 amniotic fluid samples obtained no more than 24 hours before delivery, using a critical ratio of 2.0 even with longer sample delivery internal (24-72 hours) there was no difficulty in relation to 8 out of 48 lower ratios, when the ratio was at least 2.0.

Cedard et al (1973) estimated L/S ratio in 185 amniotic fluid samples obtained during the week preciding delivery, including 170 samples obtained from 3 days before delivery. No R.D.S. detected in relation to 2.0 ratio, with intermediate value incidence was 12%, but its incidence with low ratio (0.5 or less) was as higher as 64%.

Lemons and Jaffe (1973) using charring and visual interpretation technique for L/S ratio measurement, in their series found the higher incidence of R.D.S. despite the ratio being greater than 2.0.

Donald et al (1973) reported 3.7% false positive results, which were associated with maternal diabetes and or birth asphyxia. Negstaff and Bromhem (1973) using densitometry in their series of 108 predelivery tests found only one false positive result.

Goldstein et al (1974) using 8.0 as the critical L.S.A.R. ratio reported a single false positive result in a series of 400 tests.

Using either visual assessment or densitometry in 100 cases, Morrison et al (1974) found that L/S ratio of more than 2.0 indicated safely mature lungs.

Whitfield and Sproule (1974) found that in 466 cases L.S.A.R. was more than 2.0, but R.D.S. occurred in 3 of babies (all recovered) 2 of which were born to diabetic mothers and one was anaemic due to Rhesus incompatibility. Four fifth of the babies associated with dangerously low-pre-delivery ratio (1.5) developped usually sever R.D.S. and about half of them died.

20% of those with intermediate ratio (1.5-2.0) developed moderate respiratory distress and only 1 out of 13 babies died. This finding supported the critical L.S.A.R., ratio 2.0.

Roux et al, (1974) showed that although there is a linear relationship, critical L/S ratio of 2.0 by densitometry, differ numerically from the corresponding critical L/S ratio value of 3.0 by visual assessment.

Kenisten et al (1975) also expressed that the densitometric ratios were having lower values than those of planimetric values.

Kenisten et al (1975) further reported L/S ratio measurement in 193 cases. Samples were obtained 72 hours before delivery. No R.D.S. was detected with ratio value of 2.0 but with intermediate values (1.5) the incidence was 13%.

Dubring and Thompson (1975) found the higher incidence of R.D.S. despite the ratio being greater than 2.0 and associated with series of Lemons and Jaffe, in both series together there were 12 instances of R.D.S. (2 fatal) among 205 cases with L/S ratio of more than 2.0 but 7 of the effected babies were born to diabetic mothers, 3 had sever Rhesus disease (one died and another died from R.D.S. following urgent delivery because of placenta praevia) with lower ratios. 11 out of 17 babies in these two series developed R.D.S.

In a series of 135 amniotic fluid samples obtained within 48 hours of delivery, Olson et al (1975) concluded L/S ratio by molar method and found it to be 3.5, since no R.D.S. was detected in 82 cases with higher values but increasing incidence of this complication was associated with lower values.

They concluded that R.D.S. occurred in 6 out of 35 babies (17% with no deaths) associated with ratios between 2.0 and 3.5 and in 9 out of 13 babies (69%, with 5 deaths) with ratio between 1.6-2.5. All 5 babies associated with predelivery ratio of less than 1.5 died from R.D.S. (100%). It should be noted that an increased risk of R.D.S. in neonates with low Apgar score can occur despite mature L/S ratio (Curs et al, 1976).

Rome et al (1976) using L.S.A.R. technique for L/S determination, took 2.0 to be critical value because only one baby developed R.D.S. (mild) with higher pre-delivery L/S ratio (2.1).

Tiwari and colleagues (1979) further concluded that ratio of 2.0 always indicated mature foetal lung. Tiwari et al (1979) in a series of 55 cases found mean L/S ratio 0.57 at 28-30 weeks, 2.30 at 35-36 weeks, 3.02 at 39-40 weeks and 3.45 at more than 40 weeks gestation period.

O'Brien and Cefalo (1980) found the predictive value of 'mature' L/S ratio (2 or more) about 90% in normal pregnancy. But nonmature L/S ratio (less than 2) may predict R.D.S. only in about 50% of cases. The accuracy rate of the L/S ratio was always highest, around 95 to 96% and a somewhat higher result of 98.78% in the late trimester delivery group, Chich-lung-Chow and Te-Lin-Lia (1981). But L/S ratio has continued to produce 94-98% accuracy in most reports (Doughlas Cunningham (1981).

In a series of 246 cases Sharma et al (1981) related L/S ratio with different gestational periods. It was 0.096 at 26 to 28 weeks 29-31 weeks, 0.818, 1.113 between 32 to 34 weeks, 2.207 between 35 to 37 weeks and 2.567 between 38 to 40 week and between 41 to 43 weeks 3.016.

FACTORS AFFECTING SURFACTANT PRODUCTION AND THEIR LEVEL IN AMNIOTIC FLUID

Predictable respiratory performance at birth depends upon a sufficiently high L-S ratio. In the critical intermediate range it is important that before taking decision concerning pregnancy management, the account be taken of factors influencing the ratio. Amongst these diabetics, Rhesus disease and acute birth asphyxia are more important.

Since it was first reported that the usual terminal rise in the L/S ratio does not always occur when the mother is diabetic or when there is sever Rheus incompatibility (Whitfield et al; 1972). Substancial evidence has been obtained to indicate that the amount of surface active lecithin in the amniotic fluid , and presumably also the amount being produced in the foetal lungs, amy be reduced when either of these complicatéons is present; there may also be an apparent failure to replenish initially adequate lung surfactant.

There is also good evidence that intrapartum or birth asphyxia may inhibit replenishment of surfactant in the foetal and neonatal lung, and less conclusive that foetal pulmonary maturity may be accelerated or delayed in the presence of certain other maternal or foetal complications.

On 400 premature and mature new born series Gluck (1972) portulated that early methylation pathway of lecithin synthesis is inhibited by acute hypoxia and acidosis leading to R.D.S. Results of Ronald et al (1973) were significant.

Gluck and Kulovich (1973) reported delayed L/S ratio maturation in a number of babies born to diabetic mothers.

Lemons and Jaffe (1973) found normal L/S ratio in a series of Rhesus disease infants while Shreyer et al (1974) found normal L/S ratio, but Polishuk et al (1974), Whitfield and Sproule (1974), Mukherjee et al (1974), Marois et al (1974) found subnormal L/S ratio in babies born to diabetic mothers.

Early surfactant production pathway fails rather than terminal active synthesis of dipalmitoyl lecithin, so steep fall in L.S.A.R. in association which sever Rhesus disease has not been observed after 35 weeks of gestation.

Whitfield and Sproule (1974) found normal L.S.A.R. and observed terminal rise in 150 such cases where baby was not severly affected. Freeman et al (1974) found no effect of stress upon foetal lung maturation.

Correlating the birth asphyxia, Kalbac and Hermon (1974), Duhring and Thompson, (1975) and Heniston et al (1975) found high incidence of R.D.S. in babies delivered by Caesarean section. This supports the view that respiratory distress is more likely to follow abdominal than vaginal delivery.

Dyson et al (1975) found generally normal L/S ratio in 148 samples from 71 patients with diabetics. There was a falling trend in 14 out of 35 samples with an associated increase in perinatal asphyxia and mortality. But Heniston et al (1975) found subnormal L/S ratio in babies born to diabetic mothers.

A study made on Rhesus disease, Duhring and Thompson

(1975) found normal L/S ratio except two severly affected foetuses with 'false positive' L-S ratio which resulted due to instrauterine blood transfusion.

Opposite to the view of Dyson et al (1975) of low L/S ratio between 37-42 weeks gestation in small for dates foetus, Sproule (1975) revealed higher L.S.A.R. values in pregnancies associated with preclempsia, essential hypertension or retarded foetal growth to the statistical significance only when the baby was small for dates.

Dyson et al (1975) observed significant pulmonary maturation acceleration in conditions of intrauterine hypoxia, placental insufficency, maternal vascular disease, pre-edempsia and repeated placental abruption.

Chiswick (1976) and the Berkowitz et al (1976) found considerably low incidence of R.D.S. in the deliveries associated with rupture of membranes as compared to control group. And increase in L/S ratio was shown.

The effect of labour on production of surfactant in the foetal lungs has not yet been adequately studied. While Craven et al (1976) reported fluctuating amniotic fluid lecithin levels with significant over all downward tounel during labour. Cabero et al (1976) found significantly higher labour values of L/S ratio during labour.

Whittle (1977) has recently demonstrated very variable effect of labour on the L/S ratio. By a study on 48 cases, he found that ratio increased in half, remained ratio in one third, but fell in remaining 15%. When there was rise, increase was inversing related to duration of labour. In the assessment of lung maturity in diabetes mellitus both L/S ratio and Palmitic acid concentration have proved unreliable (Dahlenberg et al, 1977; Wood et al, 1977, Mulleer and Mueback et al 1978).

Andrews and Brown et al (1979) observed considerably higher values of L/S ratio and palmitic acid concentration in diabetes patients for gestation period 35-40 weeks than control group.

Mutos et al (1979) reported altered L/S ratio in growth retarted foetus and Thomas et al (1980) found significantly higher L/S ratio in a 450 cases series of intra uterine growth retardation.

Douglass Cunningham (1981) reported 94-96% accuracy of L/S ratio in diabetic series.

MATERIAL AND METHODS

MATERIAL AND METHODS

The present study consists of 215 cases admitted or attended M.L.B. Medical College, Jhansi in the department of Gynaecology and Obstetrics during the period of July 1981 to March 1982. The cases were divided into following groups :-

Group No.	Type of cases
I	Cases of Normal pregnancy
II	Cases of abnormal pregnancy

Group I - Cases were further classified into two subgroups :-

Sub Group	Type of cases
(a)	Cases followed upto delivery
(b)	O.P.D. cases and cases which could not be followed upto delivery.

Group II - Cases of complicated pregnancy were selected including :-

(i) Prematurity
(ii) Foetal distress
(iii) Post maturity
(iv) Twins
(v) Hydrocephalus
(vi) Toxaemia of pregnancy
(vii) Ante-partum haemorrhage
(viii) Hydramnios
(ix) Heart disease

(x) Rhesus incompatibility

(xi) Diabetes Mellitus

Detailed history was taken including present, past, family, obstetric and personal histories.

Proper general, systemic and antenatal examination was done specially to judge the foetal maturity clinically.

A thorough study was made to see the stages of labour and complete examination of new born was done to assess the actual maturity after birth.

AMNIOCENTESIS

Liquor amnii collected by either of the following methods avoiding contamination :-

(1) Abdominal amniocentesis -during antenatal period

(2) Vaginal amniocentesis - during labour

(3) Collection during caesarian section.

Technique -

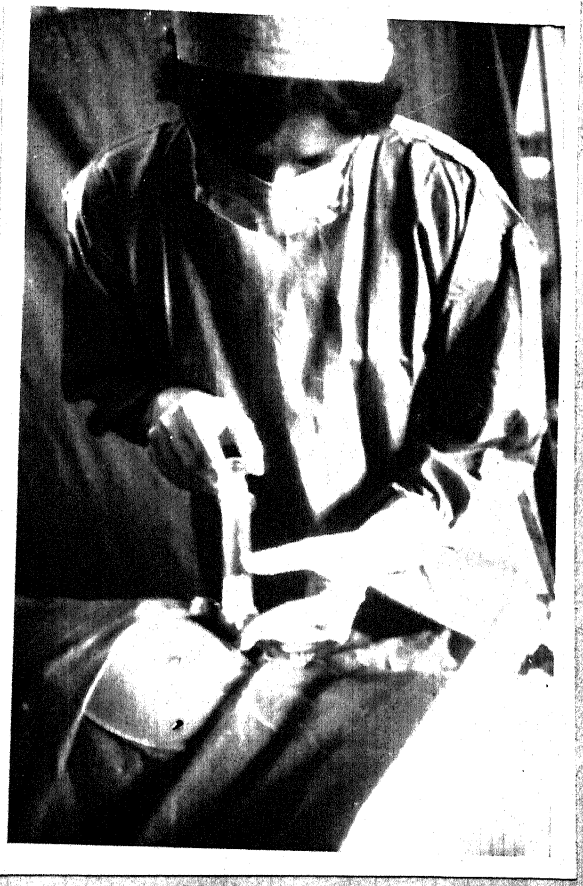
Abdominal route :-

Preliminary procedures :- Amniocentesis may be safely undertaken as an out patient procedure without pre-medication of the patient. The patient was told about the procedure and the reason for it.

Equipment and the materials required :- The tray for amniocentesis procedure contains the following :-

(i) Two 19/21 gauge wilfred Harris Spinal needles (length 3.5" - 6 or 89- 153 mm).

(ii) one pair sponge holding forceps.



Picture No. 1 . Amniocentesis (Per abdominal Route)

(iii) Sterile swabs and sponges.

(iv) Small abdominal towel with a slit

(v) Antiseptic solution and container

(vi) Sterile 5 ml. and 10 ml syringes.

(vii) Appropriate clean bottles to receive the samples.

Preparation of the patient :- The patient was asked to void urine and was made comfortable in the dorsal position on an examination table, with the head and shoulders slightly elevated to promote relaxation of abdominal muscles.

Selection of the site for amniocentesis :- The abdomen was gently palpated to determine the size of the uterus, height of fundus, foetal limbs etc. Foetal heart rate counted at this time.

The area between foetal arms and legs and of the knees of necks were the most suitable sites for insertion of the needle. Scar areas were avoided if any.

Procedure :- Having selected the puncture site, part was painted and dropped. With full aseptic precautions the needle with stilet was passed with a quick thrust through the abdominal wall and uterine wall into the amniotic cavity at the selected site. Usually a sensation of 'give' was obtained as the needle point entered the amniotic cavity. The stilet was removed from the needle, the amniotic fluid flowed through the needle. 10 ml. amniotic fluid was withdrawn.

After aspiration of the fluid, the needle was quickly withdrawn and puncture site was sealed with tincture-benjoin. The patient should remain on the table for 10 minutes. Foetal heart was auscultated again. She was told to report any fever, pain, chills, bleeding or leakage of fluid.

Following aspiration, amniotic fluid was aseptically transferred from syringe to the properly labelled sample container.

Vaginal Amniocentesis :- The transvaginal approach was applied when patient was in labour with membranes present and cervix adequately dilated.

Patient was put on table in dorsolithotomy position. Vagina and Vulva properly cleaned. Sims speculum was placed in posterior vagina and if needed cervix was held by sponge holding forceps. A 20 number L-P needle with stilet was inserted directly into the bag of waters. After removing the stilet amniotic fluid was aspirated into the syringe.

Collection during Caesarean section :- After opening the abdominal cavity, needle was inserted under vision at suitable site in uterus and amniotic fluid was aspirated with the help of sterile syringe.

Amniotic fluid was used immediately or kept at -20°C for storage if delay was expected.

STANDARD AND CHEMICALS

Standard - Lecithin and sphingomyelin were obtained from V.P.Chest Institute, New Delhi and kept at - 20°C.

Chemicals - All the reagents were analytical grade (A-R) or guranteed reagents (G.R)

1. Silica Gel - G
2. Chloroform
3. Methanol
4. Normal saline (0.9%)
5. Acetic acid
6. Perchloric acid (60%)

Reagents :-

1. Standard Phosphorus :- Concentration 0.025% mg/0.5 ml. 0.2197 grams of pottassium dihydrogen phosphate (KH_2PO_4) was dissolved in water and made upto one litre. A few drops of chloroform were added.

2. Metol (P-methylaminophenol sulphate), 1 gm in 100 ml of 3% solution of sodium bisulphite.

3. Ammonium - Molybdate solution :- 7.5 gms was dissolved in 200 ml. of water, 100 ml of @10 NH sulphuric acid was added and made upto 400 ml with water.

EXTRACTION OF PHOSPHOLIPIDS FROM AMNIOTIC FLUID

Modified method of Gluck et al (1971) was used and L/S ratio measured by molar - method.

Fresh amniotic fluid or that stored at - 20°C was centrifuged to remove cells and sediments 3000 at r.p.m. for 5- 10 minutes.

5 ml. of supernatant amniotic fluid was extracted with equal volume of methanol (5 ml) and 2 volumes (10 ml) of chloroform.

This mixture was mixed and kept for four hours with intermittent shaking.

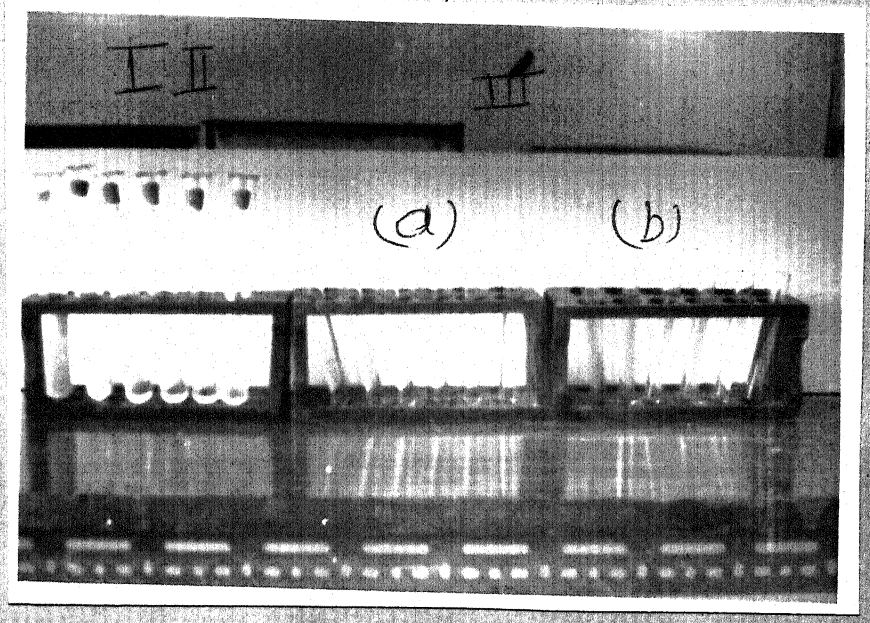
The lower clear layer, containing phospholipids dissolved in chloroform, was seperated in a beaker and supernatant again extracted with 2:1 chloroform : Methanol mixture. To extract most of the phospholipids this process was repeated thrice.

Now all these sperated samples were mixed with an equal amount (volume) of 0.9% normal saline in a separating funnel for four hours, so as to separate proteins and other sediments which precipitate as a slummy layer in between the two solutions.

The lower chloroform layer containing phospholipids was drawn into a beaker very carefully without disturbing the intermediate slummy layer. This solution was evaporated almost to dryness on water bath. For separation of individuals phospholipids, the total extracted phospholipids were dissolved in known amount of chloroform (1ml.).

THIN LAYER CHROMATOGRAPHY

Preparation of slurry for T.L.C. :- 30 gms silicagel mixed with 100 ml of distilled water containing 0.03 M $NaHCO_3$, in a conical flask and shaken riskly for 30 seconds. This



Picture No. 2. **Extraction of Phospholipids**

(i) Extraction with Chloroform and Methanol

(ii) Extraction with Normal saline

(iii) Quantitative analysis

(a) Lecithin

(b) Sphingomyelin

homogenous slurry should be immediately poured for spreading as the binder hydrates and sets within 2-3 minutes.

Preparation of the Plate :- In order to prepare a satisfactory plate the slurry must be spread evenly over the whole plate surface.

Standard size plate in T.L.C. was 20 x 20 cm. transparent glass plate of 5 mm thickness. Such plates were best spread with one of the commercially available T.L.C applicator containing spreader , fealer (gauge) and leveler.

After setting the plates, gate was fixed gauge at 0.25 mm thickness and slurry fed into the spreader which was then drawn along set of plates in a single smooth motion.

On completion of the spreading the apparatus was left for 5 minutes for layer to 'set'. In this time layers containing binder usually change their appearance from shiny wet to a dry mottle surface which indicated that gypsum had been formed.

Layer thickness :- In general layers of 0.25 - 0.30 mm thickness were used. This figure refers to the gap under the spreader (thickness of gel coating overglass plates).

Drying the plate :- The plate was dried by standing overnight at room temperature. Drying simply removes the water or other solvent used to form the slurry, and leaves the plate still containing a certain amount of water which is chemically bound.

Activation of plate :- Activation involved drying the plate at an elevated temperature, usually 110.130°C for 1-2 hours.

Active plate usually pick up water rapidly from the atmosphere and even breathing on the plate was sufficient to change degree of activity greatly. So activated plate was usually reactivated immediately before use by a further heating for 30 minutes at 110°C temperature.

Cleaning plate for re-use :- Soap or non-abrasive detergent with water was adequate for this purpose. A final rinse with distilled water followed by vertical draining and drying should ensure that plate was ready for re-use. Before coating the gel plates were cleaned with cotton soaked in acetone to remove any traces of lipoidal material over the plate.

Applying the sample :- The sample applied by micropippette Use of applicator plate ensure evenly spaced spots 1 cm apart and and 2.0 cm up from the edge. 0.02 ml. of extracted phospholipid from individual amniotic fluid samples applied with intermittent drying so that spot area was not more then a few mm in diameter.

Marking of plate :- Standard lecithin, sphingomelin and were marked by needle or pencil tip at the top of the plate with amount of quantity used.

Apparatus for T.L.C. :- Blown glass tank with [illegible] upward bowing was used so that the vertical plate will stand at an angle to the horizontal solvent surface. plate should be erect and solvent solution calm and quiet.

Solvent :- Chloroform : Methanol : Acetic acid : Distilled Water were taken in the ratio of 25 : 15 : 4 : 1

For the two plate tank as in the present study, 90 ml solvent was used. The solvent was made to run upto 17 cm height on plate from base, which used to take about 2-3 hours.

The plate was taken out and left to dry for 30-40 minutes at room temperature with a fast draught, till no solvent smell remained.

Visualisation :- The dry chromatogram was placed in a dry tank containing crystals of Iodine which rapidly volatilise to purple vapours. A tank was kept permanently for this purpose.

Lipid compounds absorb iodine reversibly to produce brown spots on a faint yellow background. On removing the plate from the tank, the colour fades as the iodine evaporates and this may be hastened with a steam of air.

Recovery of compounds from plate :- The sample spots, those corresponding in height to the spots of standard lecithin and sphingomyelin were encircled by needle. After complete evaporation of iodine, the spots were scrapped off one by one help of spatula. The material was collected on butter paper, and then transferred into the test tubes seperately for further determination.

DETERMINATION OF PHOSPHOLIPIDS IN LECITHIN AND SPHINGOMYELIN FRACTIONS OF PHOSPHOLIPID :-

This was done by modified method of Marinetti (1962). Silica gel scraped was directly digested with 1 ml. of 60% per choloric acid (Misra 1960) . Test Tubes were kept on hot plate till it becomes clear. Few drops are distilled water were added and again the mixture hoild for 2-3 minutes, to convert pyrophospholipids into inorganic phosphorus.

Then total amoun was made to 10 ml by addition of distilled water. One ml of each ammonium molebdate and metal reagents were added and it was kept for ½ hours.

Known standard made by addition of 0.5 ml. standard phospholipid solution to which 9.5 ml. of distilled water , were added. 1 ml of ammonium molbdate/1 ml metol was also added.

Control was prepared by taken 10ml. distilled water including 1 ml of metol and 1 ml of ammonium molybdate reagents.

Calorimetery :- The silica gel was allowed to settle down by centrifugation . Supernatant was used for measuring absorbance at 625 nm. using red filter in Calorimeter.

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OBSERVATIONS

OBSERVATIONS

Amniotic fluid samples from 215 cases were analysed for L/S ratio determination, including 85 cases of abnormal pregnancy. The cases were divided into two groups.

Table No. I

Showing Distribution of cases

Group	Type of cases	No.of cases	Percentage (%)
I	Cases of normal pregnancy	130	60.465
II	Cases of abnormal pregnancy	85	39.535
	Total	215	100.00

Group I cases were further subdivided into following subgroups :

Table No.II

Showing subdivision of Group I cases

Sub Groups	Type of cases	No.of cases	%
A	Cases followed upto delivery	80	61.538
B	O.P.D. Cases and cases which could not be followed upto delivery.	50	38.462
	Total	130	100.00

FIG. NO.2
DISTRIBUTION OF CASES

Group I: Normal Pregnancy Cases
'A'- Cases followed up.
'B'- Cases not followed up.

Group II: Abnormal Pregnancy cases.

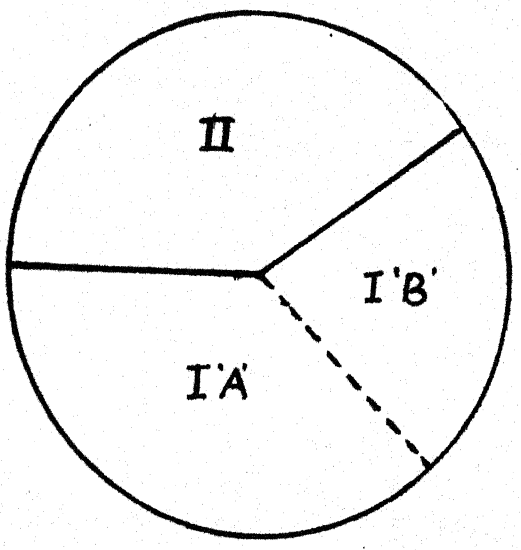
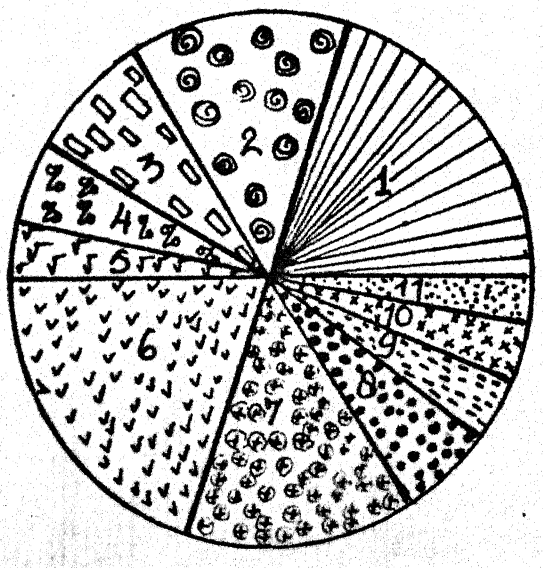


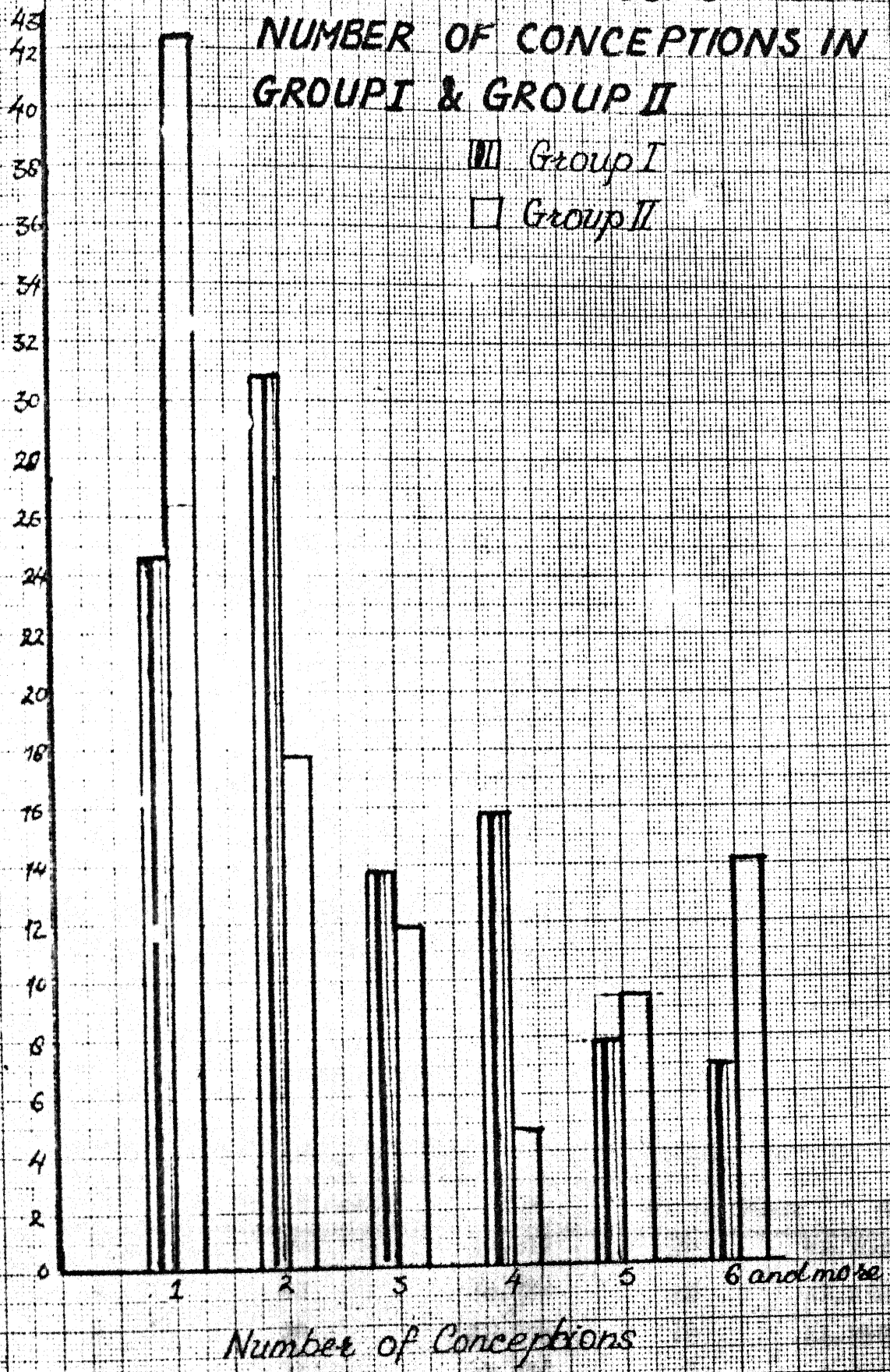
FIG. NO.3
DISTRIBUTION OF GROUP II CASES



1. Prematurity.
2. Foetal distress.
3. Post maturity.
4. Twins.
5. Hydrocephalus.
6. Toxaemia Pregnancy.
7. A.P.H.
8. Hydroamnios.
9. Heart disease.
10. Rh-incompatibility.
11. Diabetes.

FIG. NO. 4

DISTRIBUTION OF CASES ACCOPDING TO NUMBER OF CONCEPTIONS IN GROUP I & GROUP II



All cases were distributed according to the age of the mothers. It is evident from the table No.II that the most of the Group I cases were between age of 21-25 years (44.615%). But in group II (abnormal pregnancy cases), most of the women were between the age of 16-20 years (45.882%). In Group II 12.943% mothers were in 36-40 years aged as against 3.846% of normal cases in this age-group. Fig. No.2 shows distribution of cases.

Table No.III

Distribution of cases according to age.

Age (Years)	Group I		Group II	
	No.of cases	%	No.of cases	%
16 - 20	30	23.076	39	45.882
21 - 25	58	44.615	22	25.882
26 - 30	28	21.540	10	11.764
31 - 35	9	6.923	3	3.549
36 - 40	5	3.846	11	12.943
Total	130	100.000	85	100.00

Table No.IV

Distribution of cases according to number of conceptions :-

Gravida	Group I		Group II	
	No.of cases	%	No.of cases	%
1	32	24.615	36	42.352
2	40	30.770	15	17.650
3	18	13.846	10	11.765
4	21	16.154	4	4.705
5	10	7.692	8	9.411
6 and more	9	6.923	12	14.117
Total	130	100.000	85	100.000

The cases in our study ranged from primigravida to 9th gravida. In study 21 cases (9.767%)of total were gravida, 6 and more and were considered as one group of grandmultipara.

In group I maximum cases were second gravida (30.770%). But in group II the commonest group was of primigravida (42.352%). It is also evident from Fig.No.4.

Table No.V

Distribution of cases according to the period of gestation.

Period of gestation (weeks)	Group I		Group II	
	No.of cases	%	No.of cases	%
< 24	2	1.540	-	-
25- 26	3	2.308	-	-
27 -28	6	4.615	1	1.116
29 -30	4	3.076	3	3.529
31 -32	8	6.153	6	7.069
33 -34	12	9.230	9	10.588
35 -36	9	6.922	20	23.539
37 -38	33	25.381	19	22.352
39 -40	53	40.775	20	23.539
40 -42	-	-	2	2.352
43 & more	-	-	5	5.882

Table No.VI

Route of amniocentesis	No.of cases	%
Per abdomen	138	64.186
Per vaginum	46	21.395
During Caesarean section	31	14.419
Total	215	100.00

FIG. NO. 5

ROUTE OF AMNIOCENTESIS AND MODE OF DELIVERY.

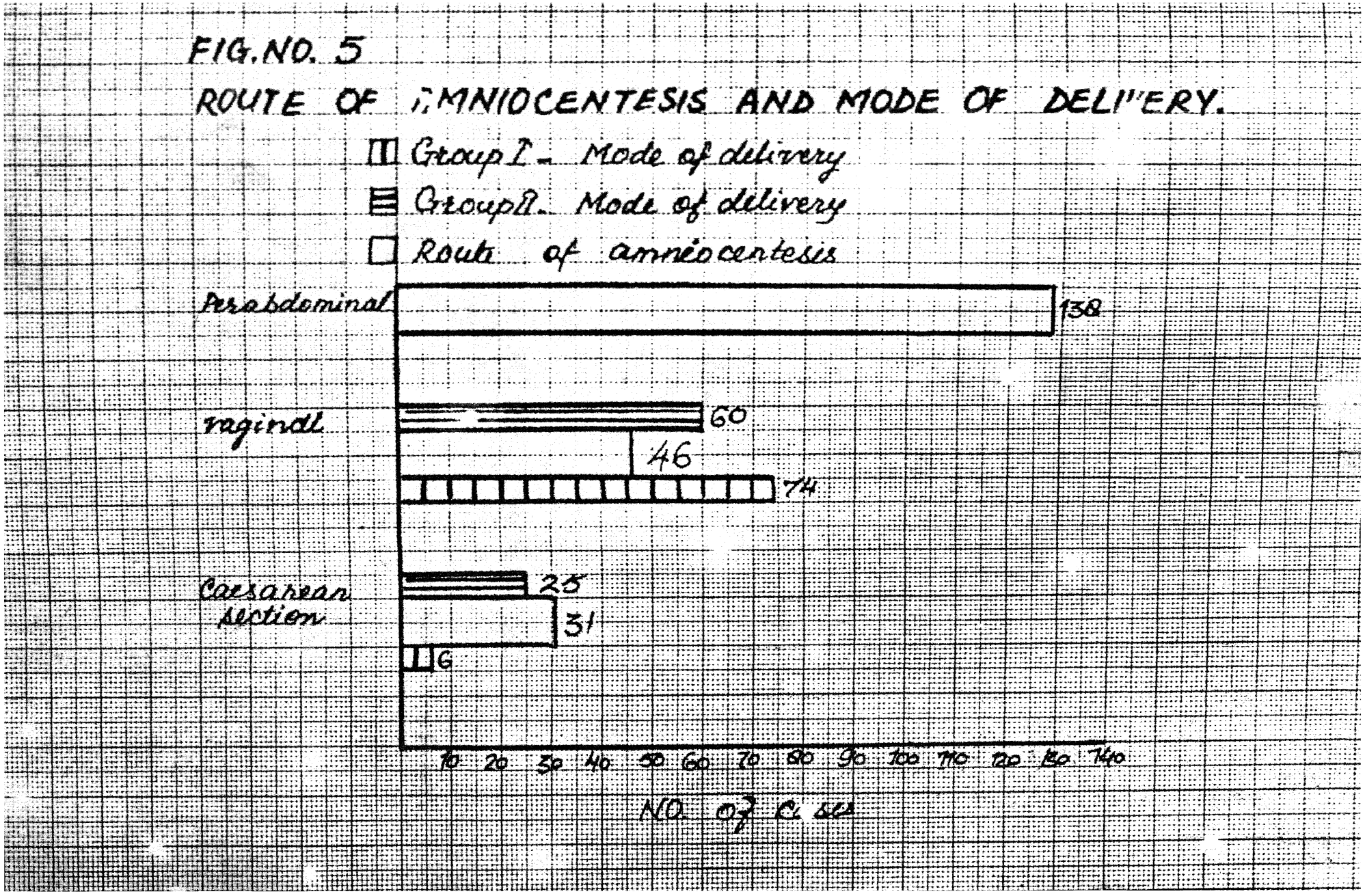


Table No.VI shows distribution of cases according to route of amniocentesis. The maximum samples were obtained by per abdominal route (64.186%). (Fig No.8)

Table No.VII

Distribution of cases according to mode of delivery

Mode of delivery	Group I		Group II	
	No. of cases	%	No. of cases	%
vaginal	74	92.500	60	70.588
Caesarean section	6	7.500	25	29.412
Total	80	100.000	85	100.00

Of the total 215 cases, 134 had vaginal delivery and 31 had caesarean section. Amongst which 25 (29.412%) caesarean sections were in group II against 6 (7.500%) cases of group I. (Fig No.5).

LECITHIN - SPHINGOMYELIN LEVELS AND L/S RATIO IN GROUP I CASES :-

A total of 130 cases were studied and L/S ratio levels levels are shown in scatrogram. No.6.

FIG. NO. 6 SCATTEROGRAM

L/S RATIO VALUES AT DIFFERENT PERIODS OF GESTATION IN GROUP I CASES

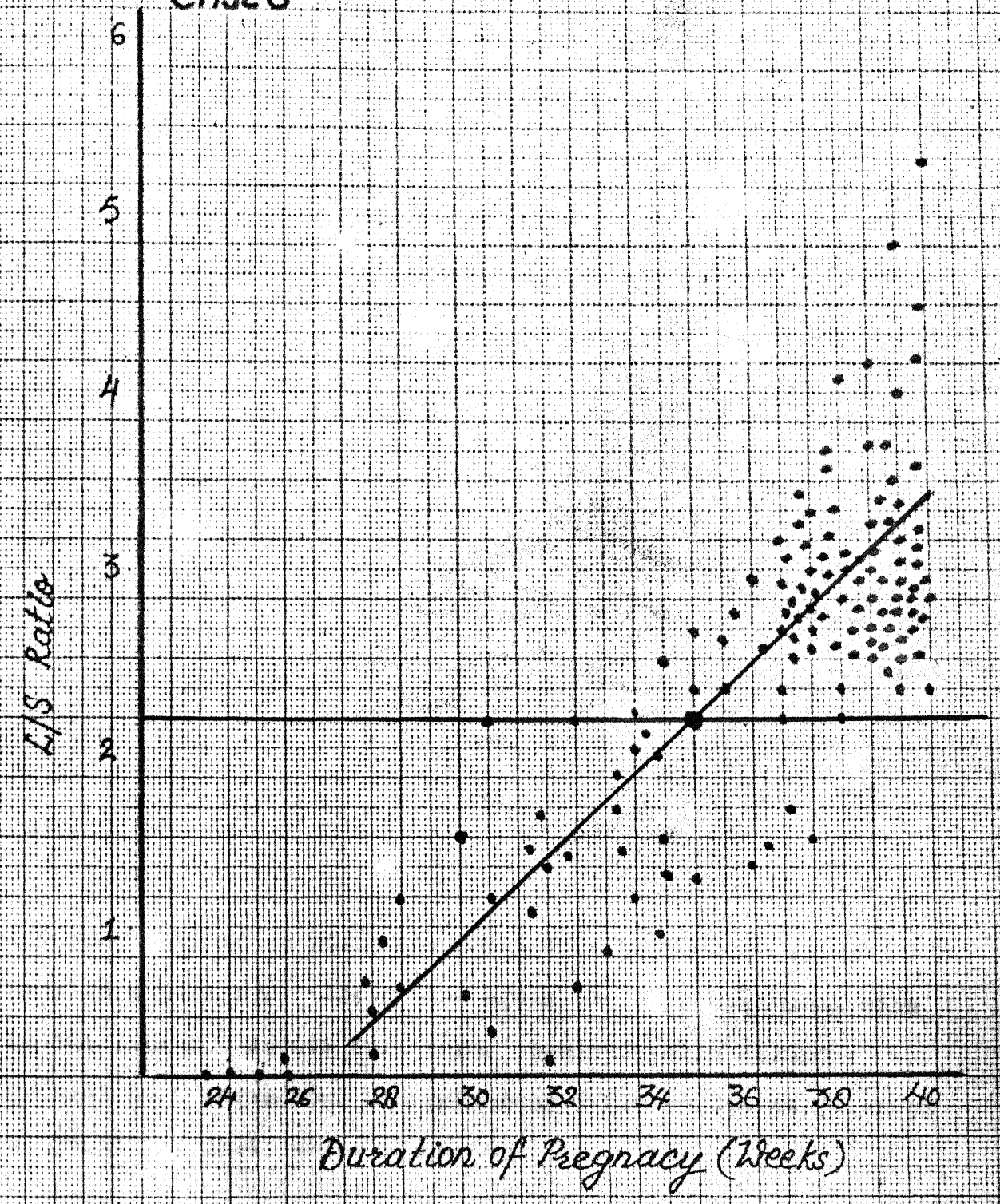


Table No.VIII

Lecithin and Sphingomyelin levels at different gestational periods in group I.

Weeks of gestation	No.of samples	Mean L	Range	Mean S	Range
<24	2	3.000	1-5	57.500	52-63
25-26	3	6.380	3-6	51.662	44-57
27-28	6	8.062	3-18	42.590	28-62
29-30	4	19.400	9-30	38.561	30-45
31-32	8	24.080	14-40	31.754	18-50
33-34	12	29.750	23-36	29.052	16-40
35-36	9	53.000	30-60	26.337	22-30
37-38	33	53.665	32-65	23.910	14-32
39-40	53	53.080	27-68	20.282	12-30

Fig No.8 shows minimum, mean and maximum lecithin values.

The minimum values of lecithin (3.00) were observed in early weeks of gestation, while sphingomyelin showed maximum values (57.500) during the same period. There was gradual rise in lecithin levels with the advancing pregnancy upto 34 weeks. Then a sudden surge of lecithin levels was observed (29.750 at 33-34 weeks and 53.000 at 35-36 weeks gestation period). After that again there is a gradual rise in the lecithin levels till term. Sphingomyelin showed gradual fall throughout the pregnancy (from 57.500 at 24 weeks to 20.282 at 39-40 weeks of gestation period).

FIG. NO. 7

MEAN VALUES OF L, S AND L/S

⊟ Lecithin (L)] Scale - 1 Sq = 1

⊟ Sphingomyelin (S)]

⊟ L/S Ratio] Scale 10 Sq = 1

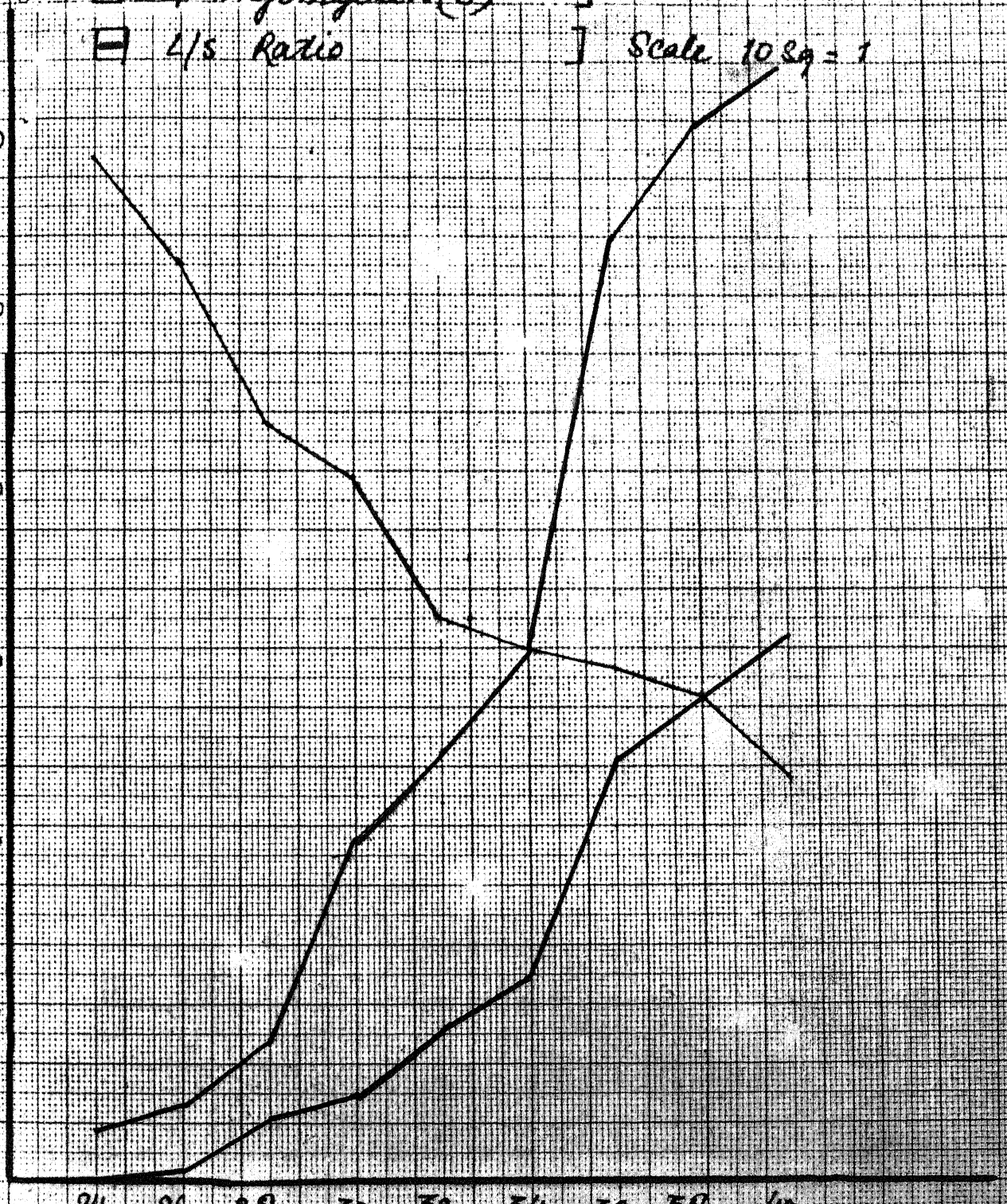


FIG. NO. 8

LECITHIN LEVELS AT DIFFERENT GESTATIONAL PERIODS

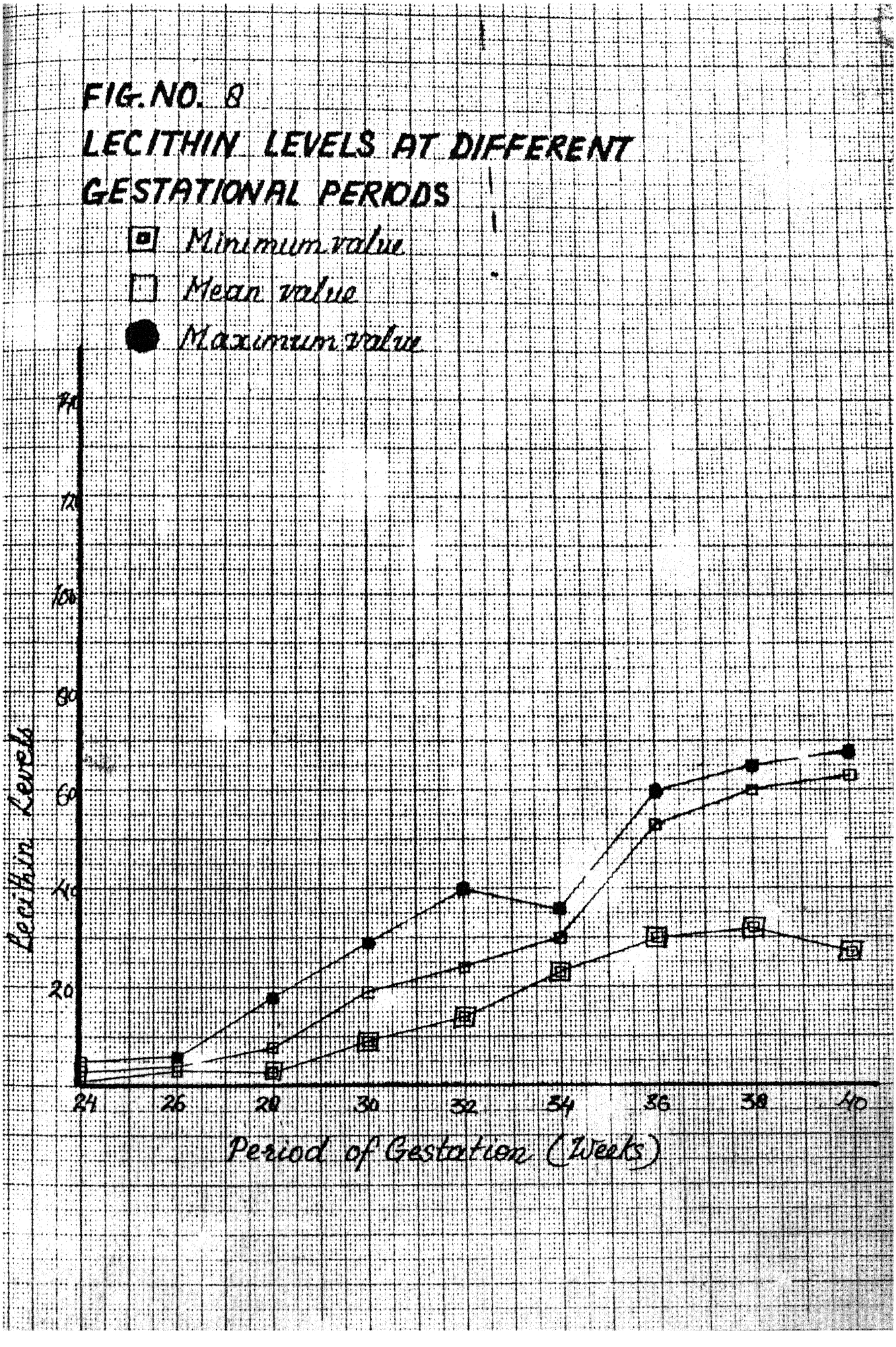


FIG. NO. 9

L/S RATIO IN GROUP I CASES

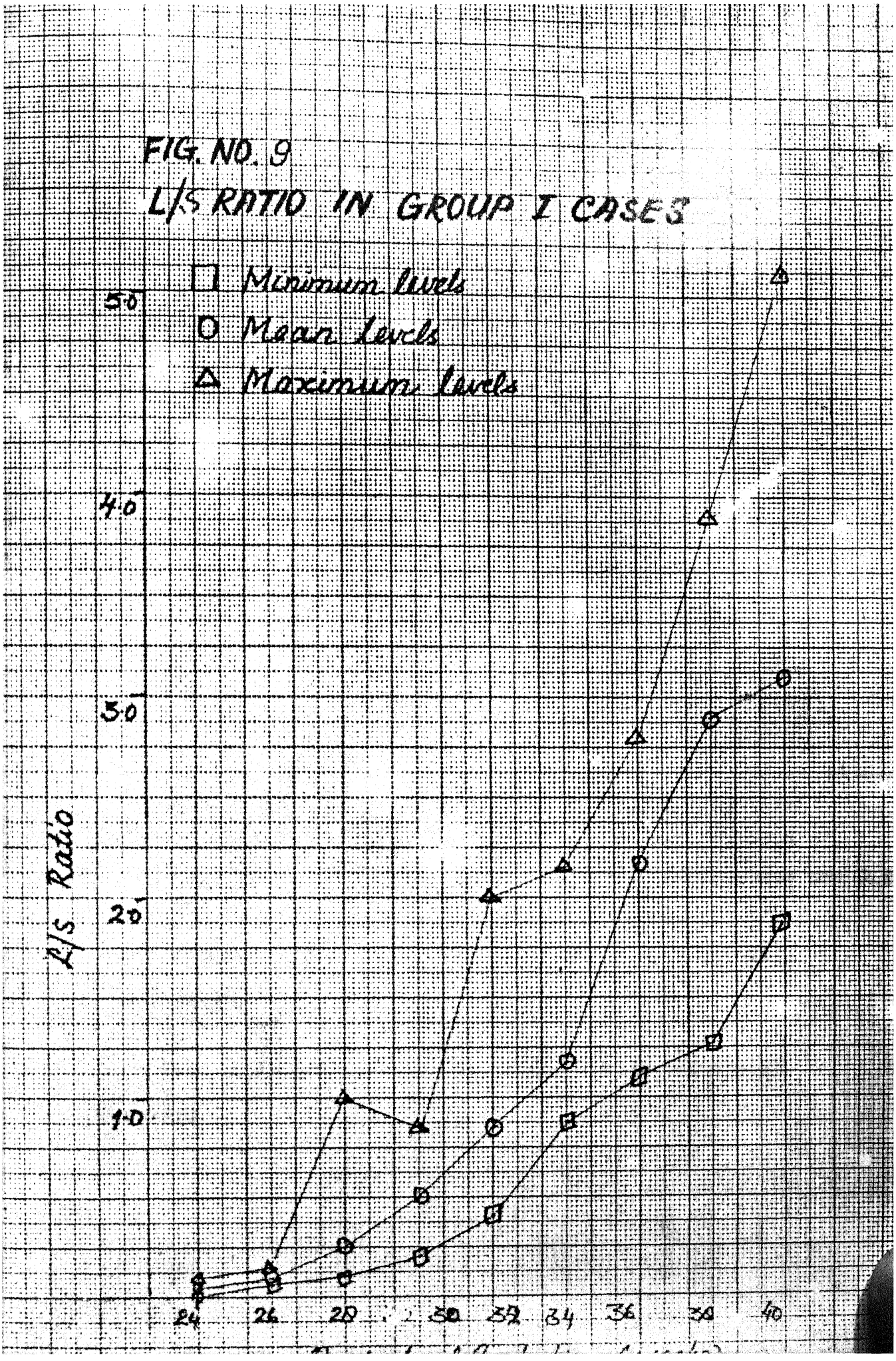


Fig No.7 shows Mean values of Lecithin, Sphingomyelin and the L/S ratio.

Table No. IX

Showing rise in L/S ratio with advancing gestation period.

Gestation period (weeks)	No.of samples	Mean L/S ratio	Range of L/S ratio	Rate of Rise
< 24	2	0.055	0.015-0.096	
25-26	3	0.098	0.055-0.150	0.043
27-28	6	0.350	0.036-1.000	0.252
29-30	4	0.500	0.200-0.720	0.150
31-32	8	0.849	0.400-2.000	0.349
33-34	12	1.180	0.722-2.168	0.331
35-36	9	2.186	1.100-2.288	1.006
37-38	33	2.418	1.290-3.908	0.232
39-40	53	3.086	1.969-5.130	0.668

It is evident from the table No.IX that the mean L/S ratio was 0.055 before 24 weeks gestational period, 0.350 at 27-28 weeks, 0.849 at 31-32 weeks, 2.186 at 35-36 weeks and 3.086 at term. It can be seen that the ratio is showing constant rise and the maximum rise was observed again at 35-36 weeks gestation period.

Fig No.9 shows minimum, mean and maximum values of L/S ratio.

FIG. NO. 10

PERCENTAGE OF L/S RATIO IN GROUP I

□ < 2·0

▨ ≥ 2·0

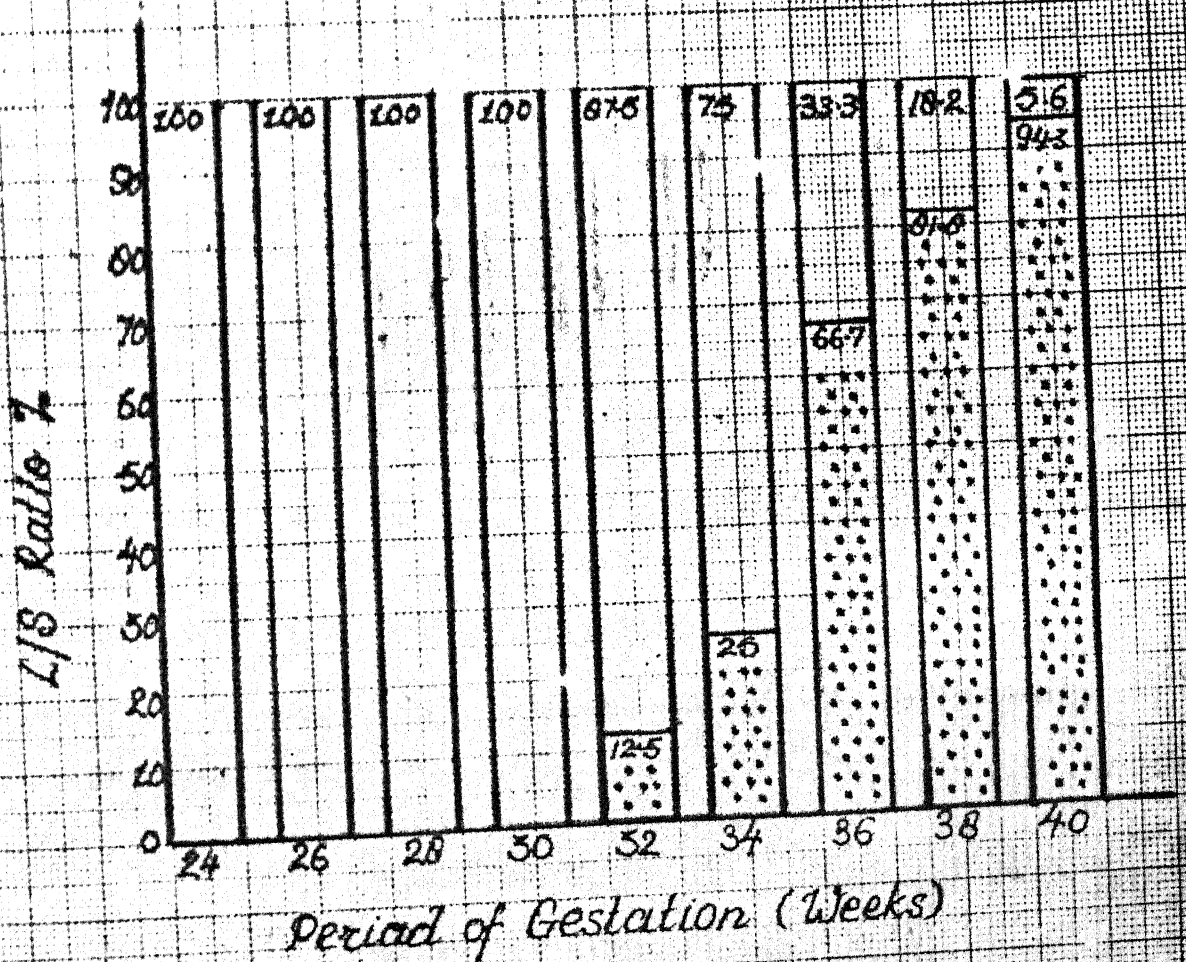


Table No.X

L/S ratio distribution in different gestation period

Gest. period weeks	No.of cases	L/S ratio 0.5	0.6-1	1.1-1.5	1.6-2.0	%<2	>2	%>2
< 24	2	2	-	-	-	100.00	-	-
25-26	3	2	-	1	-	100.00	-	-
27-28	6	3	1	1	1	100.00	-	-
29-30	4	1	1	2	-	100.00	-	-
31-32	8	-	2	2	3	87.50	1	12.50
33-34	12	-	-	1	8	75.00	3	25.00
35-36	9	-	-	1	2	33.33	6	66.67
37-38	33	-	-	1	5	18.18	27	81.82
39-40	53	-	-	-	3	5.66	SD	94.34
Total	130	8	4	9	22		87	-

From table No.X, one can see that although there is a significant progression of higher L/S ratio with advancing gestation, there is small proportion of values less than 2.0 at term (5.66%). Fully one third of (33.33%) of the values were less than 2.0 at 35-36 weeks. No value exceeded 2.0 prior to the 30th completed gestation weeks (100%).

Fig. No.10 shows the percentage of L/S ratio in Group I cases.

FIG. NO. 11 SCATTEROGRAM
RELATION OF L/S RATIO WITH
BIRTH WEIGHT

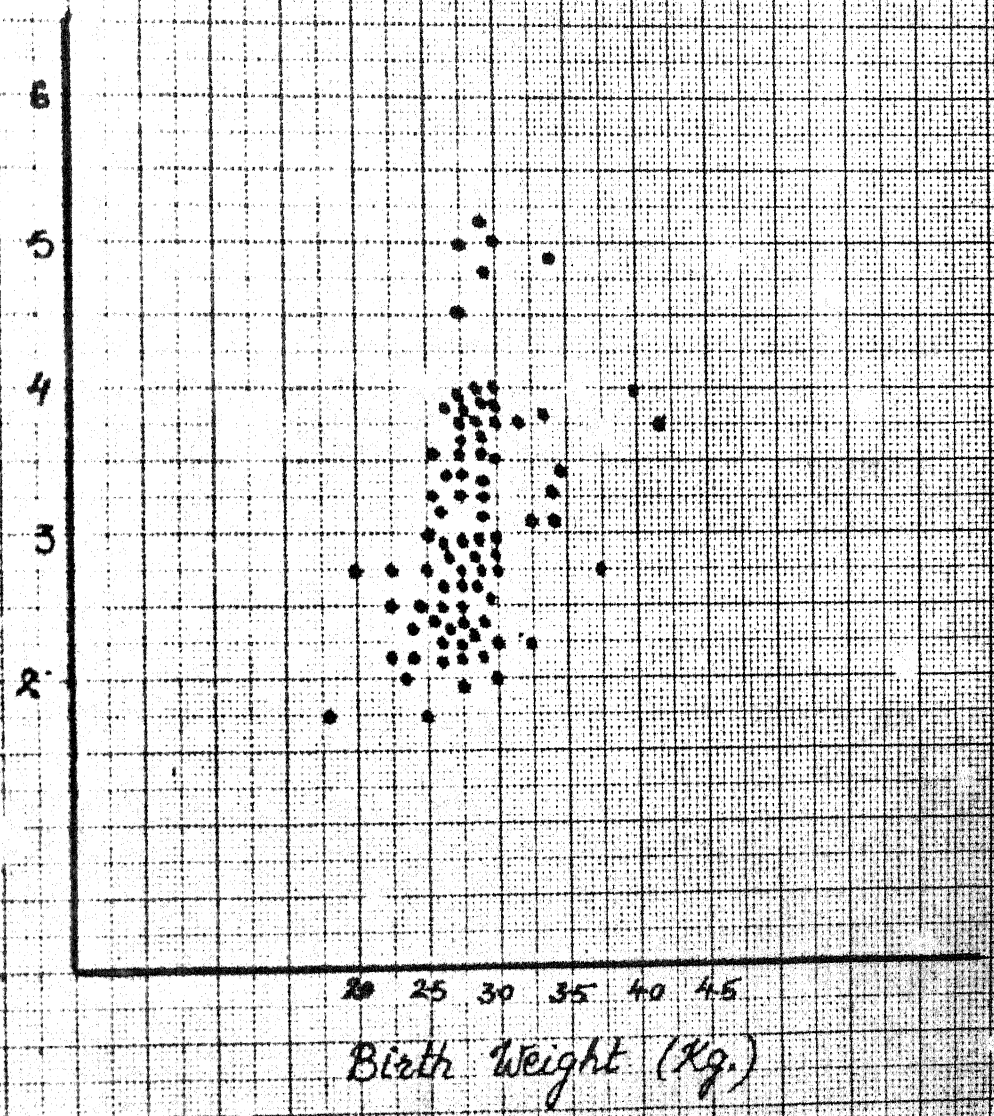


Table No.XI

Relation of L/S ratio with birth weight

	Birth-weight (K.G.)						
	< 2.0	2.1-2.5	2.6-3.0	3.1-3.5	3.6 & more	Total	%
No. of cases	2	10	57	8	3	80	100
L/S ratio							
< 2.0	1	2	1	-	-	4	5
2.1-3.0	1	8	28	1	1	39	48.75
3.1-4.0	-	-	23	6	2	31	38.75
4.1& more	-	-	5	1	-	6	7.50
%	2.5%	12.5%	71.25%	10%	3.75%	100%	

Table No.XI shows the study of 80 cases who were followed up and were between 38-40 weeks of gestation period when liquour was obtained for analysis.

Marked correlation between L/S ratio and new born birth weight was observed. Maximum cases (71.25%) had birth weight 2.6-3.0 K.G. and L/S ratio 2.1-3.0 (48.75%). There were only 5% cases with L/S ratio less than 2.0 and weight less than 2.0 Kg (2.5% cases).

The correlation between L/S ratio and birth weight is also evident from the fig.No.11.

Table No.XII

Relation of L/S ratio with R.D.S. ratio

	L/S ratio					
	< 2.0	2.1-3.0	3.1-4.0	4.1 & more	Total	Outcome
No.of cases	4	39	31	6	80	-
R.D.S						
No	2	39	31	6	78	-
Mild	2	-	-	-	2	revived
Mod	-	-	-	-	-	-
Sever	-	-	-	-	-	-

Above Table shows incidence of R.D.S. in group I cases who delivered in our hospital. Out of 80 babies 78 were normal (97.5%) till discharge from hospital. 2 neonates (2.5%) developed mild R.D.S. but recovered. The weight was 1.900 and 2.500 K.G. and L/S ratio was 2.0 and 1.960 respectively. Hence, mortality was nil in this normal pregnancy group I cases.

GROUPII (ABNORMAL PREGNANCY GROUP)

A total of 85 samples were analysed from cases showing abnormality in mother or foetus and all were followed up through delivery till discharge.

It is evident from the table No.XIII that 51.76% of the cases were associated with the affection of the foetus because of its defective maturity, well being or developement. Out of which 20% were premature and 14.18% were of foetal distress.

FIG. NO. 12 SCATTEROGRAM
L/S RATIO WITH DURATION OF PREGNANCY
IN GROUP II CASES

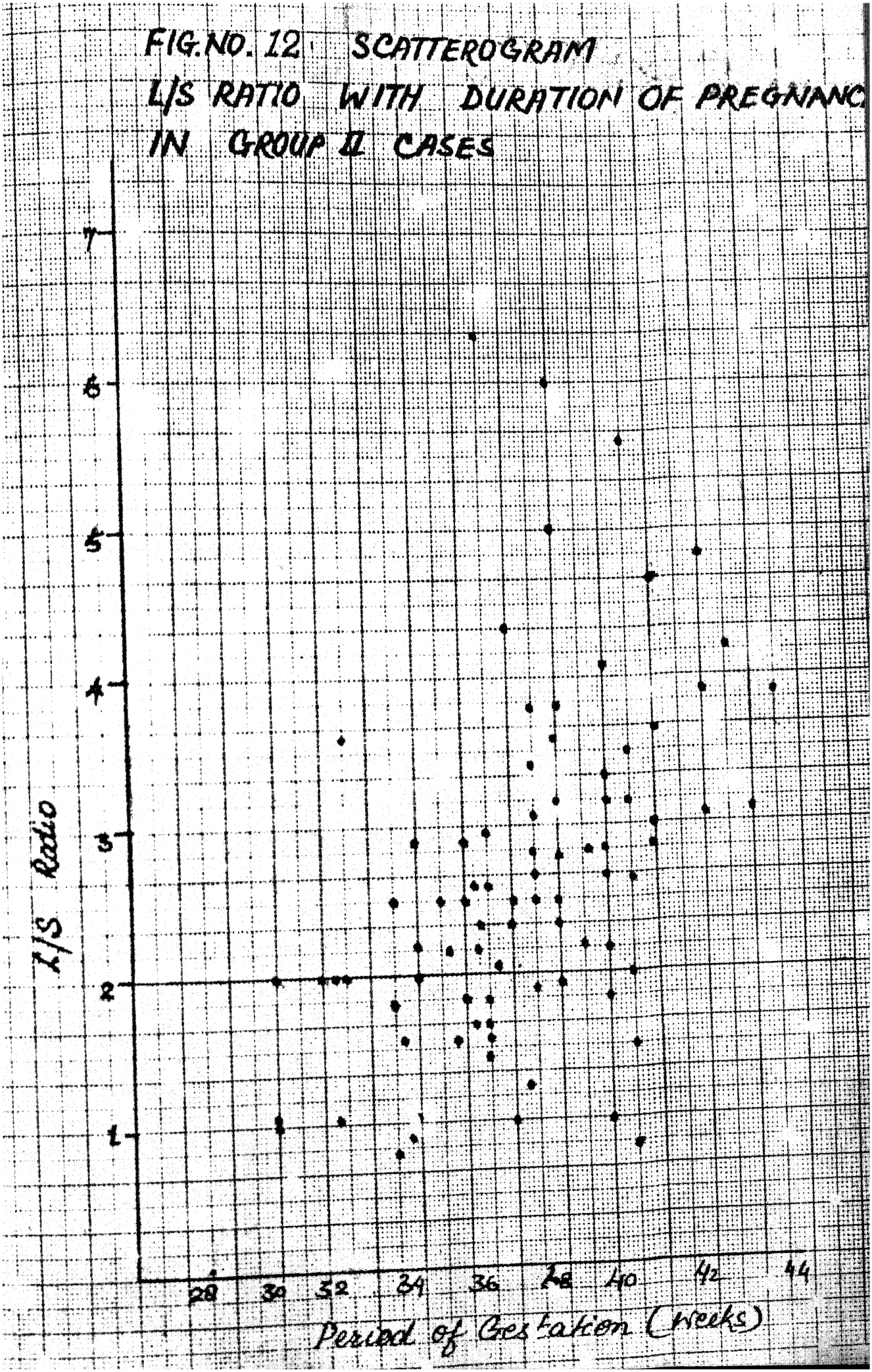


Table No. XIII

Distribution of Group II cases

S.No.	Type of cases	No. of cases	%	Foetal/maternal
1.	Prematurity	17	20.00	Foetal
2.	Foetal distress	12	14.18	51.76%
3.	Post-maturity	7	8.22	
4.	Twins	5	5.28	
5.	Hydrocephalus	3	3.52	
6.	Toxaemia of pregnancy	15	17.65	Maternal
7.	A.P.H.	13	15.28	48.24%
8.	Hydramnios	4	4.70	
9.	Heart disease	4	4.10	
10.	Rh-incompatibility	3	3.52	
11.	Diabetes	2	2.35	
	Total	85	100.00	100.00

48.24% of the total cases were associated with complications of the mother. The largest group comprised of the toxaemia of pregnancy 17.65% and A.P.H. 15.28% probably due to the place of study being a referal centre.

Fig. No.3 shows distribution of cases.

The F/S ratio in group II cases is shown Fig. No. 14 (Scattogram).

Table No.XIV

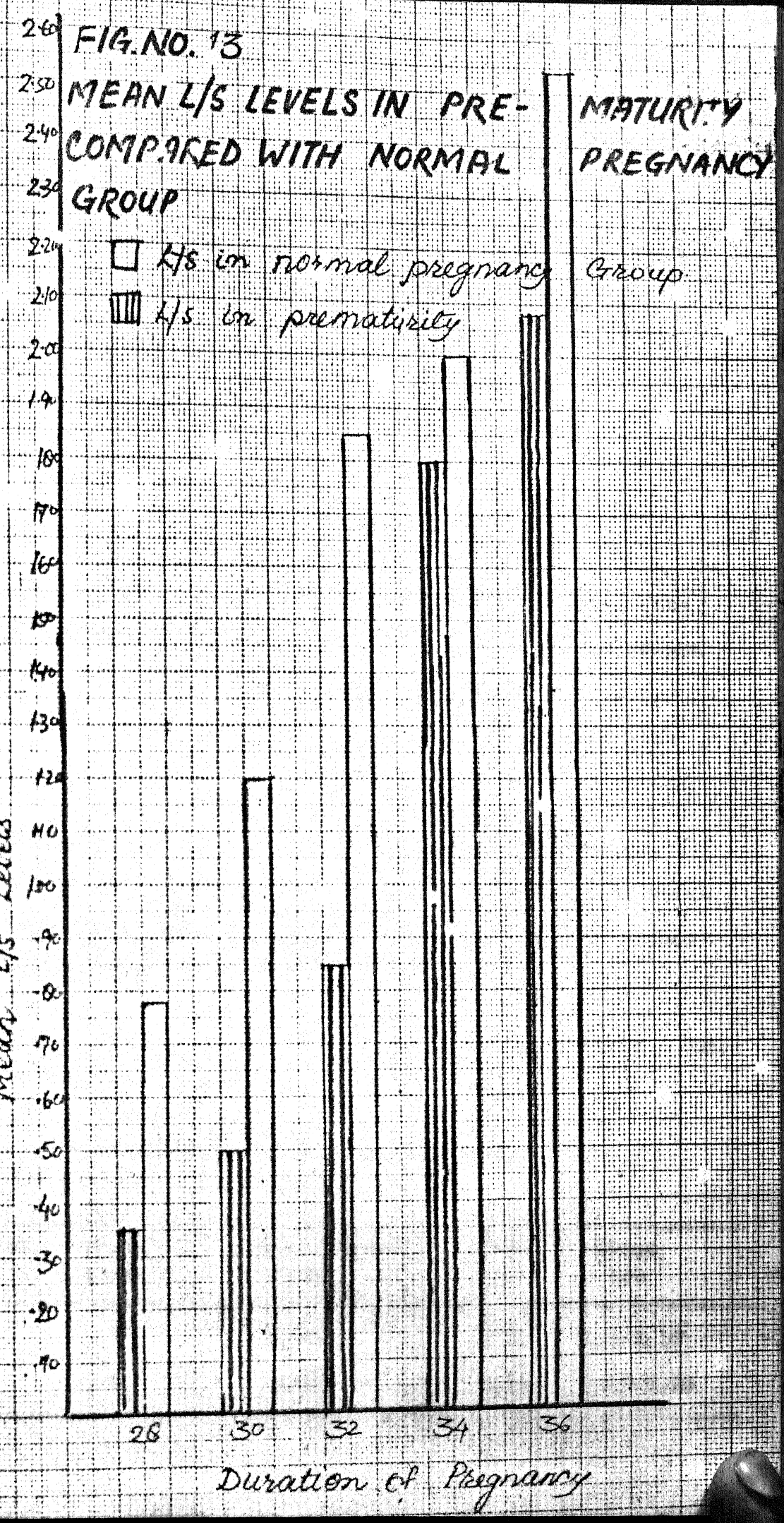
PREMATURITY

Neonate	Gest. period weeks	Weight (K.G)	L/S Ratio	Clinical state		Outcome
1	28	1.28	0.78	Sever	R.D.S.	Died
2	30	1.40	1.00	Sever	"	"
3	30	1.60	1.40	Mod	"	"
4	32	2.20	2.00	Mild	"	Survived
5	32	1.90	2.10	Mod	"	"
6	32	1.70	1.40	Sever	"	Died
7	32	2.40	2.08	-		Died of septicaemia
8	34	2.10	2.00	Mild	"	Survived
9	34	2.00	2.24	-		"
10	34	1.90	2.50	Mild	"	"
11	34	1.55	0.90	Sever	"	Died
12	34	1.85	2.70	-		Survived
13	36	2.20	4.81	-		"
14	36	2.10	2.70	Mild	"	"
15	36	1.95	1.53	Mod	"	"
16	36	2.60	2.90	-		"
17	36	2.20	2.70	Mild	"	"

A series of 17 cases was studied. The cases were of normal pregnancy who passed into labour after 28 weeks gestation and where delivery could not be checked. Amniotic fluid samples were collected during labour. The values of lecithin, Sphingomyelin weight and L/S ratio with its range are shown in table XIV.

FIG. NO. 13

MEAN L/S LEVELS IN PRE-MATURITY PREGNANCY COMPARED WITH NORMAL PREGNANCY GROUP



These premature infants weighed between 1.28 to 2.60 K.G. birth weight. All infants with except one who died due to Septicoemia , L/S ratio 2.0 or more (9cases i.e.53%) though 3 cases had mild R.D.S. with birth weight less then 2.0 K.G in 2 cases and one had moderate R.D.S with birth weight 1.9 K.G. One death in this group was due to septicoemia.

In cases where L/S ratio was less than 2.0 (8 cases that is 47%), 4 cases had sever, 2 had moderate and 2 mild R.D.S. All babies with sever R.D.S in this group could not be saved. One baby with moderate R.D.S died with birth weight 1.60 and L/S ratio 1.4. Other infant with moderate R.D.S. had birth weight 1.65 KG and L/S ratio 1.53 was revived with difficulty. All cases of mild R.D.S remained well.

FOETAL DISTRESS :- A series of 12 cases were studied. Amniotic fluid was collected from the mother where foetal heart was more than 160 or less then 100 or irregular with or without excessive foetal movements.

In four of the cases the liquor was miconium stained. The results were as follows :-

Table No.XV

L/S ratio in foetal distress

Gest. Period (weeks)	No.of cases	Mean weight (K.G.)	Mean L/S	Range L/S
37-38	3	2.70	2.06	1.0-3.19
39-40	9	2.80	3.26	2.00-4.28

Out of 12 cases, in 4 cases babies were unaffected (33.33%) in remaining 8 cases, 4 (33.33%) had mild R.D.S., 2 had moderate R.D.S. (16.66%) and 1 had sever R.D.S. (8.33%). One baby was still birth with L/S ratio in 5 cases was less than 2.0 and in 7 cases was more then 2.0 as is evident from Table No.XVI.

Table No.XVI

R.D.S. in foetal distress

	L/S Ratio		No.of cases Total	%	outcome
	< 2.0	> 2.0			
No.of cases	5	7	12	100.00	-
R.D.S. :-					
- No	2	2	4	33.33	-
-Mild	2	2	4	33.33	Revived
-Mod	-	2	2	16.66	"
-Sever	-	1	1	8.33	Expired
Still birth	1	-	1	8.33	Dead

POST MATURITY :-

Post mature cases included in this study were 7 in number, 2 with 42 complete gestational weeks and 5 with gestational period of more then 42 weeks. The weight of all the newborns was more then 3.00 K.G. All had L/S ratio more then 3.0 and incidence of R.D.S was nil (See Table No.XVII)

Table No.XVII

Gest. period (weeks)	No.of cases	Mean weight (K.G.)	Mean L	Mean S	Mean L/S	Range L/S	R.D.S
41-42	2	3.26	66.80	19.32	3.42	3.15-3.86	Nil
43 & more	5	3.74	69.00	16.24	3.90	3.60-4.21	Nil

Twins :- 5 cases of twin pregnancy were studied. Out of 10 neonates, 4 died of R.D.S. who had less than 2.0 K.G. weight.

Table No. XVIII

Neonate	Preg. (weeks)	Wt.of newborn K.G.	L/S ratio	R.D.S.	Final outcome
1	32	1.55	1.6	Sever	R.D.S. Died
2	32	1.60	1.6	"	" "
3	34	1.85	1.8	Mode	" Survived
4	34	1.65	1.8	Sever	" Died
5	36	1.80	2.18	Mild	" Survived
6	36	2.20	2.18	Nb	" "
7	36	2.00	2.70	Nb	" "
8	36	2.25	2.70	Nb	" "
9	38	2.60	2.40	Nb	" "
10	38	1.60	2.40	Sever	" Died

TOXAEMIA OF PREGNANCY :- A total of 15 cases who had blood pressure over 130/140 mmHg with or without albuminuria were included in this series. 3 cases were of eclempsia and had associated hypertension.

Table No.XIX

L/S ratio in toxaemia of pregnancy

Gest. period (Weeks)	No.of cases	Mean weight	Mean L/S	Range L/S
31-32	1	1.55	3.68	-
33-34	2	2.10	1.60	0.80-2.40
35-36	10	2.80	2.27	1.42-6.30
37-38	2	2.75	5.05	3.98-6.02

	RANGE OF L/S RATIO									
	1	1.1-2.0	2.1-3.	3.1-4	4.1-5	5.1-6	6.1 & more	No	%	Outcome
No.of cases	1	4	6	1	1	1	1	15	100.00	-
R.D.S :-										
- No	1	4	4	1	1	1	1	9	60.00	-
- Mild	-	1	-	-	-	-	-	1	6 .66	Revived
- Mode	-	1	2	-	-	-	-	3	20.00	1 died
-Sever	-	-	-	-	-	-	-	-	-	-
still birth	1	1	-	-	-	-	-	2	13 .33	dead

Table No.XXI

ANTEPARTUM HAEMORRHAGE (A.P.H.) :-

Gest.Period (weeks)	No.of cases	Mean L/S	Range of L/S	WEIGHT		
				2.0	2.1-3.0	3.1 & more
29-30	1	2.03	-	1	-	-
31-32	-	---	-	-	-	-
33-34	1	1.98	-	-	1	-
35-36	2	2.25	2.0 -2.50	-	2	-
37-38	5	2.76	1.9-3.96	-	3	2
39-40	4	3.00	0.94-5.60	-	3	1

(Table XIV & XV)

Two new borns were still births with L/S ratio 0.80 and 1.42. Three babies developed moderate R.D.S., and amnog them 1 died, who was delivered to an eeclamptic mother by L.S.C.S. with L/S ratio 1.96 other 2 had L/S ratio between 2.1-3.0 and were revived. 2 had mild R.D.S. and survived.

A total of 13 cases of A.P.H. were studied. 3 had chronic bleeding P/V samples were collected per abdominally at the time of delivery or during caesarean section.

As it is shown in Table No.XXII that 2 cases had still births with L/S ratio 0.94 and 1.4 and weight 1.68 K.G. and 2.3 K.G. Three cases had moderate R.D.S. and the neonates died. In one case L/S ratio was less than 2.0 while in others it was more than 2.0 the case was of accidental haemorrhage with toxaemia of pregnancy. In two cases, there was mild R.D.S. One case had sever R.D.S. L/S ratio was 1.5 as is shown in Table No.XXII.

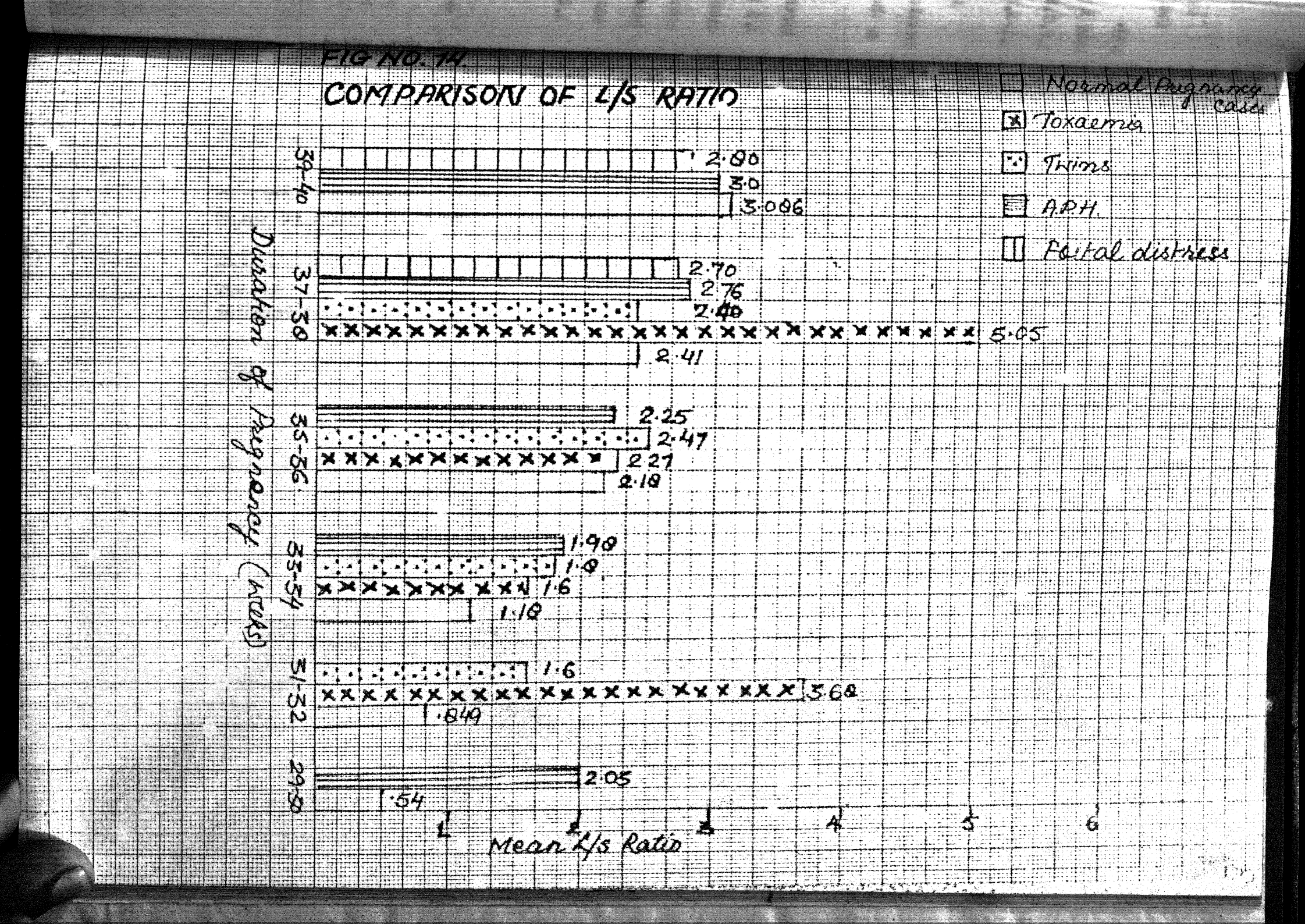
Table No.XXII

Incidence of R.D.S. in A.P.H.

	L/S ratio							
	I	1.1-2.0	2.1-3.0	3.1-4.0	4.1 & more	Total No	%	Outcome
No.of cases	1	3	5	2	2	13	100	-
R.D.S								
-No	-	-	2	1	1	4	30.76	-
-Mild	-	-	1	1	1	3	15.38	-
-Mode	-	1	2	-	-	3	23.07	Expired
-Sever	-	1	-	-	-	1	7.69	"
Still birth	1	1	-	-	-	2	15.38	Dead

FIG NO. 14

COMPARISON OF L/S RATIO



Disease	Gest. weeks	No. of cases	Mean L/S	Range L/S	Mean weight	R.D.S	Outcome
Hydrocephalus	35-36	1	1.60	-	3.200	Mode	Died
	37-38	-	-	-	-	-	-
	39-40	2	2.20	1.90-2.50	3.480	-	Died
Heart Disease	37-38	4	2.15	2.0 -2.50	2.680	-	-
Rhesus incompatibility	37-38	1	2.34	-	2.80	-	-
	39-40	2	2.56	2.20-2.92	2.82	-	-
Hydramnios	37-38	3	2.10	1.86-2.50	2.85	mild	Recovered
	39-40	1	2.08	-	2.70	-	-
Diabetes	37-38	1	3.90	-	3.64	Mild	Recovered
	39-40	2	2.85	2.7-3.0	2.90	-	-

HYDROCEPHALUS :- 3 cases of hydrocephalus were studied in 35-36 and 39-40 weeks gestation period. One newborn with L/S ratio 1.60 and birth weight 3.4 Kg. developed moderate R.D.S. and died after 28 hours. One baby with L/S ratio 1.90 was delivered by needling to drain C.S.F. was still birth. Another newborn with L/S ratio died after 6 hrs of cardio-respiratory failure, the C.S.F. was drained by needling during second stage of labour to facilitate the normal vaginal delivery.

HEART DISEASE :- 4 cases of heart disease were studied in 37-38 weeks of gestation period. Mean L/S ratio was 2.15, no R.D.S was detected.

RHESUS INCOMPATIBILITY :- 3 cases of RH incompatibility were studied. One at 37-38 gestation weeks and other 2 at 39-40 weeks. Mean L/S ratio was 2.34 and 2.56 respectively. No baby developed R.D.S. Antibody titre in 2 cases was nil. But in one case it was 1.8.

HYDROAMNIOS :- Among 4 hydroamnios cases, one baby with L/S ratio 1.80 at 37-38 weeks of gestation period developed R.D.S. (mild) but recovered.

DIABETES MELLITUS :- Out of 3 cases under study one baby developed mild R.D.S. but was revived. L/S ratio was 3.90 and birth weight 3.85 Kg at 37-38 weeks gest.period. Mother was on insulin. Other two babies remained well.

D I S C U S S I O N

DISCUSSION

The quality of perinatal life is well known to be dependent on genetic input, maternal environment the gestational age and birth weight attained, and it is further modified by intrepartal and neonatal events. More recently, as one of the first major advances made in perinatal medicine, there has been the further recognition that foetal biologic maturity , apart from gestational age and weight , is also essential to a safe transition through crisis of birth and new born period. This is specially throughout foetal pulmonary maturity.

As the perinatologist has gained better control of the timing of birth, both by delaying and hastening it based on increased foetal concerned, it has become critical to have a reliable perinatal prognostic index of foetal maturity. This is specially so in cases were complicating factors are involved such as previous caesarean section, Rh sensitization maternal diabetes mellitus, toxaemia of pregnancy and uncertain gestation period based upon irregular or inaccurate menstrual reporting. The practice of early delivery especially were date of delivery is uncertain , might increase perinatal mortality. In order to avoid this mishap a variety of 'foetal maturity tests' have been developed.

A test for foetal maturity should be quick and accurate.

In recent years a number of components of amniotic fluid have been noted to change progressively during pregnancy and accoddingly have been intensely investigated as indication of foetal maturity. Amniotic fluid is now easily accessible and amniocentesis is relatively simple and safe technique to help the issue further.

Many intrauterine tests to detect foetal well being have been described including by parotal diameter by ultrasound (Donald 1969) and Ise et al 1971), distal femoral epiphysis (Murdock 1959), Nile blue dye test (Brosens et al 1966, Sharma et al 1970) amniotic fluid bilirubin (Mandel baum et al 1967) and Creatinine (Pitkin et al 1967). But all these tests have been recommended as indices for determining the gestational age and or foetal weight. But the most essential is the physiological maturity that of the foetal lungs.

The foetal lungs make a small contribution of amniotic fluid (Goodlin and Rudolph 1970) and are the source of some of its constituents including Phospholipids (Scarpelli 1967, Nelson 1969). Amniotic fluid phospholipids and in particular the lecithin to sphingomyelin ratio appeared to provide an index of foetal maturity (Gluck et al 1971) and Spellacy et al (1972).

The present study of determination of foetal lung maturity by L/S ratio was undertaken to ascertain whether such an estimation would prove helpful in determing foetal maturity as well as in reducing the incidence of R.D.S. in neonates especially in complicated pregnancies.

For the purpose of discussion total of 215 cases were divided into 2 groups.

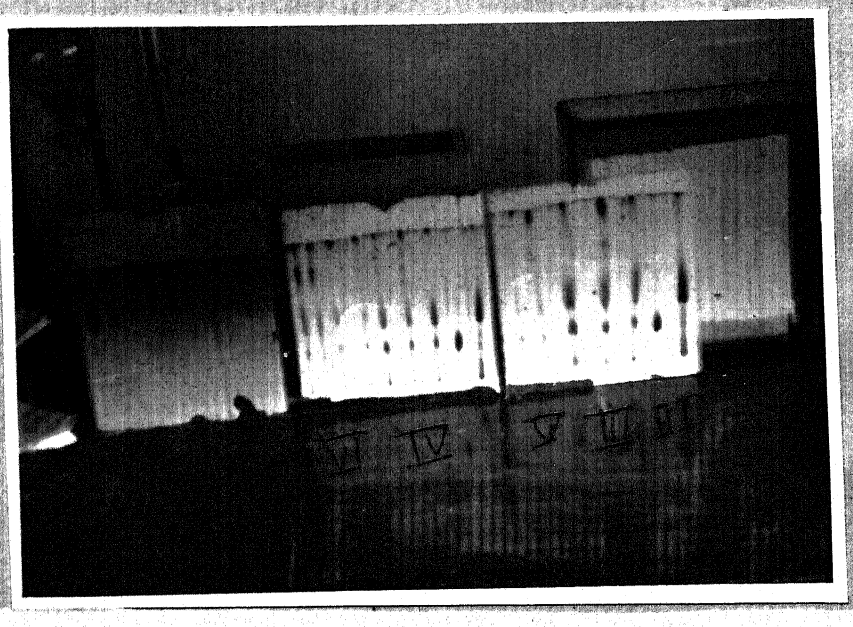
Group I - Cases of normal pregnancy
Group II - Cases of abnormal pregnancy.

Group I - A total of 130 cases were studied out of which 80 cases were followed up through delivery till discharge. Out of these 80 cases, 74 cases (92.50%) delivered vaginally. 60 cases were normal viginal delivery (75%) and 14 cases (17.50%) were delivered by forceps and 6 cases (7.50%) underwent caesarean section for causes like contracted pelvis, Cephelop-elvic disproportion and malpresentations.

The lecithin, sphingomyelin values and their ratio were studied during various periods of gestation (Table No. VIII and Table No. IX).

Bhagwanani et al (1972) noted rising values of lecithin throughout pregnancy till term. Our findings are consistent with their observations. (Fig. No. 8)

Picture No. 3. Lecithin Sphingomyelin spots on T.L.C.



(i) Standard Lecithin spot

(ii) Standard Sphingomyelin spot

(iii) Lecithin and Sphingomyelin spots on 40 weeks of gestation period.

(iv) Lecithin Sphingomyelin spots at 36 wks. of gestation period.

(v) Lecithin Sphingomyelin spots at 30 wks. of gestation period.

(vi) Lecithin incontaminitve

(vi) Lecithin and Sphingomyelin spots in contaminated samples. (blood and Meconium)

Dunn and Bhatnagar (1973) reported a gradual rise in each of these phospholipids begining at 16th week and continuing as pregnancy approaches term.

(Table No.VIII), maximum rise was observed between 35-36 weeks in our series while Bhagwanani et al (1972) observed exaggregation from 34 weeks. Bizenski (1973) recorded a similar trend in lecithin concentration.

Gluck et al (1971) saw a surge in lecithin concentration at 36th week of gestation, heralding mturity at foetal lung. Clinical interpretation was made in T.L.C. lecithin spot clearly larger than sphingomyelin marked pulmonary maturity in the foetus. (Fig.No.13). Hobbins et al (1972) could predict the respiratory outcome of newborns by studying lecithin sphingomyelin spots on T.L.C.

Their results were very similar to Gluck et al (1971). Clements et al (1972). They observed an abrupt rise in the titre of surfactant at about 35 week of gestation. This study has revealed the same pattern. (Fig.No.8).

Reverse trend was observed with sphingomyelin values (Table No.VIII). A gradual fall was seen which was also observed by Arvidson et al (1972) Brzezinski et al (1973). Both Gluck (1977) Dunn et al (1973) have reported rising values of Sphingomyelin throughout the pregnancy which was opposite for our observations. (Fig.No.7).

While an abrupt surge in lecithin level was detected at 35-36 weeks of gestation, the fall in sphingomyelin level was trhoughout our gradual in our study.

Thus it is correct to say that lecithin is the principal phospholipids of late pregnancy with a rise from 3.0 at 24 weeks to 63.08 at 40 weeks, where as , sphingomyelin appeared to be the principle phospholipid of early pregnancy with a fall in levels from 57.50 at 24 weeks to 20.282 at 40 weeks, as according to our study. (Table No.8).

L/S ratio was studied in some series of 130 cases. A general rise is in the values with increasing gestational age was detected (Fig.No.6). Estimation of the ratio between lecithin and sphingomyelin by far the most widely used and accepted approach to the measurement of the surfactant in amniotic fluid.

The mean values of lecithin, L/S ratio, sphingomyelin observed in present serées are shown in table No.8 & 9. The range of L/S ratio is shown in Table No.9. Graph No.9 shows minimum , mean and maximum values of L/S ratio.

The mean values were quite low before 26 weeks of gestation i.e .055 before 24 weeks , .098 between 25-26 weeks and .850 between 27-28 weeks.

Sharma et al (1981) have reported mean values .096 between 26-28 weeks almost in accordance to our values.

The comparison of mean values and range of L/S ratio observed by us and reported by Tiwari et al (1969) are much resembling, as shown below.

Table No.XXIV

Gest. (Wks)	Mean L/S ratio		Rate of rise		Range of L/S	
	Our series	Tiwari series	Our series	Tiwari series	Our series	Tiwari series
29-30	.500	.57	-	-	.200-.850	.45-.70
31-32	.849	.85	.349	.280	.40-2.0	.50-1.8
33-34	1.180	1.16	.331	.310	.722-4.16	.50-2.0
35-36	2.186	2.30	1.006	1.140	1.10-4.78	1.50-3.0
37-38	2.418	2.86	.232	.560	1.29-3.90	2.10-3.8
39-40	3.086	3.02	.668	.16	1.96-5.13	1.50-4.1

Values reported by Sharma et al (1981)

Table No.XXV

Gest. period (Wks)	Mean L/S ratio	Rate of Rise
26 -28	0.096	-
29-31	0.818	0.722
32-34	1.113	0.295
35-37	2.207	1.094
38-40	2.567	0.060

It is evident from table No.XXIV and XXV that our values are identical with their values with very insignificent difference. I all the series, there is definite spurt between 35-36 weeks while before and after this period of gestation there is a slow rise in L/S ratio in each series. This rises is significant as Gluck et al (1971) clerified sudden rise in lecithin levels and the L/S ratio from about 35 weeks signifies that foetal lungs are now mature and R.D.S will not occur. (Fig.No.15).

Whitfield et al (1972) stated that there is a widening range of normal values during last two months are pregnancy which considerable individual variation in both time of onset (32-37 weeks) and the rate of terminal increase.

It is clear from Table No.10 that no values exceeded 2.0 prior to 30th week of gestation. Till 34 weeks, more cases (75%) had less than 2.0 ratio while between 35-36 the pattern reversed in most of the case (66.67%) had L/S ratio more than 2.0, at term 94.34% cases had L/S ratio more than 2.0 and only 5.66% cases had values less than 2.0. Out of 3 cases who had less than 2.0 L/S ratio, 2 cases had ratio of 2.0 while one had 1.96. (Fig No.10)

Follow up study of 80 cases where amniotic fluid was obtained within 10 days of delivery (Table No.XI and XII) revealed significant results regarding new born. All the babies in this group remained well till discharged. Only two babies developed mild R.D.S and were fully and easily revived. The criteria taken for diagnosis of R.D.S & in our study were - tachypnoea, retraction and expiratory grunting.

All the respiratory difficulties were recorded. The newborns were examined clinically. Gestational age was estimated by physical characters and pregnancy data.

All babies remaining well signify that the lungs are mature. A ratio of two or more seen in majority of cases concides with the capability of neonate to thrive.

According to Whitfield et al (1972) L/S ratio above 2.0 can be regarded as safe from the view point of pulmonary function. They held a ratio in the range of 1.5 to 2.0 as an index of transitional level of pulmonary maturity with chance of R.D.S. after delivery. A ratio of 2.0 always indicate that baby born at that time may be free from R.D.S. (Gluck et al 1974), as is evident from study too. (Table No.XII).

Correlation of L/S ratio with neonatal weight was done also (Table No.XI). It was seen that higher the birth weight more was the L/S ratio. (Fig.No.11). One neonate with birth weight less than 2 Kg. developed mild R.D.S while L/S ratio in this baby was also less than 2. In another baby with birth weight 2.5 Kg. also developed R.D.S. where L/S ratio was less than 2.

In cases studied by Spellacy and Buhi (1974), L/S ratio and infant birth rate correlated significantly. No constant relationship could be established between neonatal weight or gestational age and lung maturity by Tiwari et al (1979).

Group II Abnormal Pregnancy cases

A total of 85 cases were studied and all followed up through delivery till discharge.

Prematurity :- 17 cases of group II of our study fell in this group. Samples were collected during labour. The values observed are shown in Table No.14. The gestational period was between 28-36 weeks.

8 cases (47.059%) out of this series had 2.0 L/S ratio while 9 had (52.94%) 4.0 or more . 5 neonates (29.411%) developed mild R.D.S, 3 had moderate 17.64%) and 4 were the victims of sever R.D.S (23.529%).

Since many neonatal deaths are due to respiratory disorders , the association of R.D.S. with prematurity is inevitable. R.D.S. due to progressive atelectasis of hyaline membrane disease is a leading cause of death. (Tiwar et al 1979).

In agreement with Gluck et al (1974), in our series, the severity of R.D.S. was inversily related to L/S ratio. The infants definitely had low birth weight and morbidity and mortality in inverse relation to the incidence of R.D.S. i.e higher the birth rate lesser are the chances of R.D.S. according to our results. (Tiwari et al (1979) could not establish a constant relationship neonatal weight and lung maturity. But spellacy and Buhi (1972) from significant correlation between L/S ratio and infant birth (birth) weight.

It is clear that L/S ratio of 2.0 always indicate a mature fœtal lung. For ingants in this group dead due to severe R.D.S., their birth weights were 1.28, 1.40, 1.70, 1.55 Kg. and L/S ratio were .78, 1.00, 1.20 and .90 respectively. Three cases had moderate R.D.S. with birth weight 1.60, 1.90, 1.95 Kg. and L/S ratio was 1.40 1.10, 1.53 respectively. One of this died within 24 hours while 2 were revived difficulty but survived. 5 cases had mild R.D.S. All had birth weight above 1.90 Kg. and L/S ratio 2.0 or more. and all were revived. Almost all infants who survived required intensive resustation and care.

It is evident from the study that lesser the L/S ratio more are the chances of R.D.S. Similarly, babies with higher birth weight had better chances of survival.

This study can help us in deciding the time of induction in various cases like those of mistaken dates and bad obstetric history. L/S ratio of 2.0 or more will certainly lung maturity , hence better chances of survival of neonate after birth.

Table No.XXVI

Gestation Period	Mean L/S ratio	
	Group X	Premature series
28 Weeks	0.350	0.78
29-30	0.500	1.20
31-32	0.849	1.845
33-34	1.180	2.068
35-36	2.186	2.540

The table shows the difference in L/S ratio determined during labour in premature cases as against the values of L/S ratio in normal pregnancy cases at same gestation period without labour. The volumes are definitely higher in premature labour cases. (Fig. No. 13).

The effect of labour on the production of surfactant in the foetal lungs has not yet been adequately studied. Craven et al (1976) reported fluctuating amniotic fluid lecithin levels, with a significant overall downward trend during labour. Cabero et al (1976) found significantly higher values for lecithin and L/S ratio in samples obtained at amniotomy during labour than in samples obtained before labour. Whittle (1979) has recently demonstrated a very variable effect of L/S ratio. But he also found increasing trend of L/S ratio in 50% cases. Our findings are consistently with those of Cabero et al and Whittle.

Foetal Distress :- 12 cases were studied and amniotic/lyx fluid samples collected vaginally and at the time of Caesarean section. In 41.66% cases L/S ratio was less than 2.0 while in 59.44% it was more than 2.0. 75% cases had R.D.S out of which 2 had severe R.D.S and died. 1 was still born. Total mortality rate being 33.3%.

It was observed that inspite of L/S ratio more than 2.0, 5 cases out of 7 developed R.D.S.

Donald et al (1973) besides findings particularly high incidence of R.D.S., when both predelivery L/S ratio and Apgar score were unsatisfactory , noted that 12 out of 13 ladies developed R.D.S despite a predelivery L/S ratio at least 2.0 with Apgar score less than 7, five minutes after birth.

Several authors have reported R.D.S. occuring despite L/S ratio in bbbies delivered by Caesarean section. (Kalhan and Newman (1974); Duering and Thompson, 1975; Maniston et al 1975).

In our series, 5 out of 8 cases who developed R.D.S. were delivered by Caesarean section. These cases provide example of impaired replenishment of surfactant resulting from acute if usually transient asphyxia, i.e not infrequently seen in babies delivered by caesarean section.

Post maturity :- A total of 7 cases were studied and results are shown in Table No.23. In all cases L/S ratio was more than 2.0 and incidence of R.D.S. are nil.

Our findings are consistent with Sharma et al(1961), and Tiwari et al 1979. Their values are 3.016 and 3.45 respec ively. by in our study mean L/S ratio in the gestation period was (more than 40 weeks) 3.66.

Twins :- In our series twin cases were 5 in number. Amniotic fluid samples collected during labour gestation period ranged from 34-38 we ks.

Out of the 10 new borns, 3 had severe R.D.S. with L/S ratio less than 2. One baby with L/S ratio less than 2 2 suffered from moderate R.D.S. b ut one neonate with L/S ratio 2.4 also had severe R.D.S., Birth weight being 1.6 Kg. Mild R.D.S was in one case and 3 babies remained well. Mortality rate was 60%. It was observed that infants with L/S ratio more than 2 has better chances of survival.

Toxaemia of Pregnancy :- A series of 15 cases was studied, out of which 3 cases had eclampsia. Comparatively higher mean L/S ratio values were observed than corresponding group I values. Figure No.14. Early rise of L/S was observed and hence, early lung maturation.

Dyson et al (1975) also observed significant pulmonary maturation acceleration in conditions of pre-eclampsia . 3 cases in our series had essential hypertension, and L/S ratio was 6.34 , 6.02 and 5.84. As a study by Richbers et al (1975) in his series with conclusion that among Chronic hypertensives there was a definite trend towards an early rising L/S ratio.

33.3% cases developed R.D.S., while in two cases, there was still birth. Total mortality rate was 45% . Higher incidence of R.D.S. was observed inspite of L/S Ratio more than 2.0.

ANTE PARTUM HAEMORRHAGE

This series included 13 cases of group IIout of which three had chronic bleeding per vagina.

The overall incidence R.D.S. was 53.84% (7 cases). L/S ratio was less than 2 cases. over all mortality weight was 30.07%. (Table No.XXI). It is evident from graph No.14 that the values of L/S ratio were almost corresponding to those of normal pregnancy. One case had very high L/S ratio (5.60) in which case the baby had abruptio placentae with toxoemia. There were two still births. High incidence of R.D.S. was obtained in this group despite of the L/S ratios being mature (2.0 or more).

Lemons and Jaffe (1973) and Dahring and Thompson (1975) found that R.D.S may occassionally occur despite L/S ratio greater than 2.0. In this series, such results were obtained in cases of diabetes, Rh sensitization and A.P.H.

HYDROCEPHALUS :- 3 cases of hydrocephalus were studied between 35 -40 weeks of gestation period. One was still born and the other 2 neonate death occurred due to R.D.S within 24 hours. The values in this 2 cases were less than 2.0 and in one case it was 2.50 i.e slightly on both side. But the series is too small to comment upon.

Heart Disease :- Amniotic fluid sample from 4 cases of heart disease were studied. No changing in L/S ratio was observed. As compared to the normal pregnancy group. No R.D.S. was detected in this group.

Rh Incompatibility :- Only three cases were studied and Gestation period was 37-40 weeks. Neonates were healthy and L/S ratio more then two.

Whitfield and Sproule (1974) found L/S ratio within normal range in cases, in which the foetuses was not surely affected. Lower L/S ratio were seperated in 50% cases, where foetuses were severly affected.

Lemons and Jaffe (1973) and Duhring and Thompson (1975) reported normal L/S ratio in these patients of Rh-incompatibility Our findings are consistent with all these authors. The series is very small and the field needs further exploration to give any opinion.

HYDRAMNIOS :- Only 4 cases were under study. One case of L/S ratio 1.80 developed mild R.D.S. All other babies remains well and L/S ratio was more then 2.0.

Diabetes Mellitus :- This series covered only 3 cases of Group II. L/S ratio was more then 2.70 in all cases. Our findings are corresponsent with Donald et al (1973). Scheyer et al (1974) and Dyson et al (1975) who found normal L/S ratio values. Our findings do not correspond with Whitfield and Spouris (1974) to found subnormal L/S values in diabetes.

Gluck and Kulovich (1973) also reported the delayed L/S ratio maturation in new borns to diabetic mothers. Singh et al (1974) subsequently followed by Gluck et al (1974) Mutherjee et al (1974), Kaniston et al (1975) and Morris et al (1974) also found delayed L/S ratio maturation.

But our series is very small for any conclusive results.

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SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

In the present study, lecithin and Spingomyelin levels and thier ratio (L/S) in amniotic fluid were studied in normal and abnormal pregnancies.

In total 215 samples were studied including 130 cases of normal pregnancy. Out of which 80 cases were followed up discharge. Similarly 85 abnormal cases were studied , and 68l were followed through delivery till discharges. From this study, it was concluded that-

(1) Amniotic fluid lecithin values show gradual rise throughout pregnancy upto 35 weeks, when there is a sudden spurt. The rise after that is again gradual till term.

(2) Sphingomyelin showed gradualyy declingin values through out pregnancy.

(3) lecithin is the principle phospholipid of late pregnancy while sphingomyelin appeared to be the principal phospholipid of early pregnancy.

(4) L/S ratio showed a general rise throughout pregnancy till term. The rate of rise though sustained, showed a sudden and marked rise between 35-36 weeks, thereafter the rate of rise is again gradual.

(5) The sudden rise in lecithin, and L/S ratio, values from about 35 weeks of gestation signified the maturity of foetal lung.

(6) No values of L/S exceeded 2.0 prior to the 30th week of this gestation. More than 50% cases had L/S ratio less than 2.0 upto 34 weeks of pregnancy. Thereafter a pattern reversed and 66.67% cases had more than 2.0 L/S ratio while only 33.33% had less than 2.0 L/S ratio. This trend was maintained advancing gestational age showing more than 2.0 L/S ratio in 94.45% and only 5.56% had less than 2.0 L/S ratio at term (39-40 weeks of pregnancy).

(7) All the neonates remained well in the normal group except 2. who had mild R.D.S. which were completely revived.

(8) This further confirmed the significance of L/S ratio which was 2.0 or above in almost all the cases : the level which has been claimed as safe from the view point of pulmonary function by various authors.

(9) A direct relationship was observed between L/S ratio and birth weight.

Group II (ABNORMAL PREGNANCY CASES)

According to our results the L/S ratio was 2.0 and more always indicated a mature foetal lung and hence bright chances of survival.

Prematurity :- In nearly half of cases L/S ratio was less than 2.0. Mortality rate was 35%. All had L/S ratio less than 2.0 except one who died of septicaemia. Incidence of R.D.S was 70%.

The values we re higher as c ompare to normal group at same gestation period due to patients being in labour.

L/S ratio thus can be helpful in deciding about induction of labour. Accoroing to our study labour should never be induced if L/S ratio is less than 2.0.

Foetal distress :- 75% babies developed R.D.S. and about 62.5% had (5 cases) L/S ratio more then 2.0. Mortality rate was 25% out of which 75% had L/S ratio less than 2.0.

Postmaturity :- Values of L/S in all more than 3.0 and incidence of R.D.S. were nil. This showed that level of L/S ratio is directly proportional to the foetal maturity.

Twins :- Neonates with more than 2.0 L/S ratio had very little chance of developing R.D.S. The levels of L/S ratio were higher as compared to the Group I cases due to patients being in labour.

Toxaemia of Pregnancy :- Values of L/S ratio was higher as compa compared to normal pregnancy group I in corresponding gestation period. This denotes significant pulmonary maturity accel eration.

Antepartum Haemorrhage :- In A.P.H. high incidence of mortality and R.D.S. was seen though the L/S ratio was more than 2.0 in most of these cases.

Hydrocephalus :- Slightly lower values of L/S ratio were observed.

Heart Disease :- No significant disease change was observed. In L/S ratio from that of normal pregnancy values.

Rhesus Incompatibility :- L/S ratio was not affected.

Hydramnios :- L/S ratio was normal except in 25% cases, where the baby developed mild R.D.S.

Diabetes Mellitus :- Normal L/S ratio values were observed.

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